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# The Journal of Laboratory and Clinical Medicine

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## Clinical and Experimental

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# FUNCTION OF THE ADRENAL CORTEX IN ASCORBIC ACID DEFICIENCY AND SCURVY IN THE MONKEY

CHARLES T. STEWART, M.D., ROBERT J. SALMON, M.Sc., AND CHARLES D. MAY, M.D.
MINNEAPOLIS, MINN., AND IOWA CITY, IOWA

ASCORBIC acid is present in high concentration in the adrenal gland and depletion of this vitamin follows the administration of corticotropin. If ascorbic acid is necessary for synthesis of adrenocortical hormones, a decreased elaboration of hormones might be anticipated in scurvy.

The initial approach to estimation of adrenocortical function in scurvy was to test the effect of insulin on the level of glucose in the blood in scorbutic monkeys. It was found that the dose of insulin which caused marked depression of the blood glucose in controls had very little effect in scorbutic animals. Obviously, this finding could be better interpreted as indicating increased adrenocortical function rather than decreased.[1]

It seemed necessary to verify this conclusion by a more direct measurement of hormone production by the adrenal cortex. Therefore the spontaneous urinary excretion of 17-ketosteroids as well as the rise in excretion during stimulation with corticotropin was studied in monkeys in various phases of ascorbic acid deficiency. The associated alterations in the level of eosinophils in the peripheral blood and tissue content of ascorbic acid are also presented.

## MATERIALS AND METHODS

*Care of the Animals and Arrangement of the Experiments.*—The monkeys (*M. mulatta*) were handled as described in detail in earlier reports giving the diet and manifestations of the animals.[2, 3] The essentials are recapitulated.

The animals were housed in individual metabolism cages. The average initial weight was 2.5 kilograms, and the age was estimated as 15 months. These immature animals were selected to exclude the effect of sex on the urinary excretion of 17-ketosteroids.

The monkeys were reared on dried cows' milk reconstituted with water to normal composition. The milk diet of all animals was supplemented with iron, vitamins A, D, and E, and members of the B complex of vitamins.*

To insure complete removal of ascorbic acid from the diet all milk was stored with copper sulfate and then boiled. The diet of "control" animals was supplemented with 50 milligrams of ascorbic acid by mouth daily. On this regimen "control" monkeys gained

From the Departments of Pediatrics, Colleges of Medical Sciences, University of Minnesota, Minneapolis, and State University of Iowa, Iowa City.

Presented in part before the Society for Pediatric Research, May 5, 1952.

This study was made possible by the generous support of M and R Laboratories, Columbus, Ohio. Dietary supplements were provided by the Eli Lilly Company, Indianapolis, Indiana and Distillation Products Industries, Rochester, New York, for which we are most grateful. The ACTH used in this study was generously supplied by Armour Laboratories, Chicago, Illinois.

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*About one-half of the animals received supplementary folic acid after they developed scurvy. No difference was observed in the results obtained in the group receiving this supplementary folic acid.

in weight and were maintained in good health. Animals deprived of ascorbic acid began to fail in vigor and weight gain after about a month. Consequently, monkeys deprived of ascorbic acid for 30 days or more and not yet exhibiting gross signs of scurvy were designated "ascorbic acid deficient." During the third month without ascorbic acid there was a rapid loss in weight, progressive anorexia, and a decrease in general vigor. Gross scurvy usually developed about 80 days after the exclusion of ascorbic acid from the diet. "Scorbutic" monkeys exhibited swollen tender joints, roughening of the coat, and severe wasting. As a burst of periorbital hemorrhages frequently heralded the onset of scurvy, this sign was usually used to denote the advent of gross scurvy. Two to three weeks before this sign appeared characteristic changes were present in roentgenograms of the long bones.

The monkeys were seen daily by a physician to insure recognition of illnesses other than scurvy which might effect the urinary excretion of 17-ketosteroids. The most common illnesses encountered were respiratory tract infections and dysentery and animals contracting either were excluded from the experiment until completely recovered.

An "adrenalectomized-control" animal was prepared by subjecting a "control" monkey to a bilateral total adrenalectomy in order to measure the urinary excretion of 17-ketosteroids in the certain absence of adrenocortical tissue. Fifty milligrams of ascorbic acid was continued daily. The basal milk diet of this animal was supplemented with a mixture of sodium chloride and sodium bicarbonate to provide an additional 50 milliequivalents of sodium and 30 milliequivalents of chloride per liter of milk (500 to 600 ml. were consumed daily). General vigor was normal, and the weight was stabilized slightly below the preoperative value. Careful post-mortem examination eleven months after adrenalectomy failed to disclose any remnants of either adrenal gland or any accessory adrenal tissue.

In "control" monkeys several 24-hour specimens of urine were obtained to determine the spontaneous excretion of 17-ketosteroids. The animals then received 10 mg. of ACTH* (Acthar Gel) intramuscularly twice daily for 3 days. Twenty-four-hour specimens of urine were collected and analyzed on each day of stimulation. The same procedure was followed in monkeys deprived of ascorbic acid. The first period of stimulation commenced on the thirtieth day without ascorbic acid, and subsequent periods were begun at about ten-day intervals after the start of the first period. Thus, the animals were allowed a rest of at least seven days between each period of stimulation. In an attempt to detect any alteration in the spontaneous urinary excretion of 17-ketosteroids accompanying the onset and progression of scurvy, stimulation with ACTH (Acthar Gel) was temporarily omitted in several animals after the seventieth day without ascorbic acid (average onset of scurvy, 80 days). After obtaining several urine specimens in the initial phase of scurvy, these animals were stimulated again in subsequent periods.

Determinations of the fasting level of eosinophils in the peripheral blood were performed in "control," "ascorbic acid deficient," and "scorbutic" monkeys.

In each group of monkeys analyses for liver and adrenal content of ascorbic acid were performed on specimens of tissue obtained by biopsy or immediately after the animals were killed. Thus, a comparison could be made between the functional status of the adrenal cortex and the tissue concentration of ascorbic acid.

## METHODS

*Determination of 17-Ketosteroids in the Urine.*—The 24-hour specimens of urine were collected with 5 ml. of glacial acetic acid added as preservative. A suitable amount of urine, usually about 100 ml. or one-half of the 24-hour specimen, was transferred to a

*The Acthar Gel utilized in these experiments was generously supplied through the courtesy of Dr. E. E. Hays, Medical Director, Armour Laboratories. This material is a heat-stable, long-acting form of ACTH.[4] The Acthar Gel contained 20 mg. of ACTH per cubic centimeter. This was diluted with a sterile gelatin solution to provide a final concentration of 5 milligrams of ACTH per cubic centimeter. The animals received 2 c.c. (10 mg.) of this mixture intramuscularly twice daily during stimulation. No local or generalized reactions to this preparation were encountered.

flask with a ground glass neck. An amount of concentrated hydrochloric acid equal to 10 per cent of the volume of urine under analysis was added and the mixture refluxed for ten minutes. After cooling to room temperature, the urine was extracted four times in a separatory funnel using 25 per cent of its volume of $CCl_4$ in each extraction.[5, 6] The combined extracts were washed with 20 ml. of distilled water. Stable emulsions were occasionally encountered and then, to aid in better separation, a few ml. of 2.5N NaOH were added to the aqueous phase. After allowing to stand a few minutes without shaking, the $CCl_4$ layer was withdrawn and washed with 20 ml. of 2.5N NaOH and twice with 20 ml. of distilled water. Finally, it was dried by filtering through a dry filter paper. The $CCl_4$ was distilled off in an all-glass apparatus on a water bath under vacuum. The last trace of $CCl_4$ was removed by a gentle current of air. The extracted 17-ketosteroids were dissolved in a suitable volume of 95 per cent aldehyde-free ethanol. In an animal stimulated with corticotropin, the volume used was 10 ml., but in an unstimulated animal it was usually 2.5 ml. The 17-ketosteroids were determined by the Zimmerman reaction[7, 8] according to the technique of Holtorff and Koch[9] employing a Coleman Model 14 Universal Spectrophotometer.

In a few instances when the monkey was severely scorbutic, a flocculent precipitate separated when NaOH was added to the $CCl_4$ extract. This was allowed to separate as completely as possible at the interface between the two phases. After drawing off the lower clear $CCl_4$ layer, the precipitate was centrifuged in a narrow centrifuge tube. The upper aqueous phase was poured off. The precipitate was carefully pressed aside with a spatula while the $CCl_4$ layer was added to the rest of the extract.

As it was found that contamination of the urine specimen with feces caused a falsely high value for 17-ketosteroids, only clear specimens of urine were used. In severe scurvy, diarrhea not infrequently occurs. Then, in order to obtain clear urine specimens, it was often necessary to starve the animal for twelve to twenty-four hours, giving only water, which usually caused prompt cessation of diarrhea. Very occasionally 10- to 12-hour collections were all that could be obtained as clear specimens.

*Determination of Ascorbic Acid in the Tissues.*—The liver and adrenals were analyzed immediately after removal from the animal if possible; otherwise the tissue was frozen a short time until analyzed. After weighing, the tissue was ground with clean quartz sand and a chilled freshly prepared acid mixture containing 16 per cent trichloracetic acid and 4 per cent metaphosphoric acid (3 ml. of acid to 1 to 2 Gm. of liver or 0.2 to 0.6 Gm. of adrenal). The extract was poured into a volumetric flask, and the grinding of the residue with acid was repeated three times. The combined extracts were made up to 25 to 50 ml. with distilled water according to the probable ascorbic acid content. The extract was shaken with activated carbon (Darco, G-60) filtered and the total ascorbic acid activity determined in the filtrate after the methods of Roe and associates.[10, 11]

Although blanks were run on all reagents, zero values for ascorbic acid were never found even in tissues of the most severely scorbutic animals. It is very doubtful that the trace of material reacting like ascorbic acid is actually ascorbic acid. It could not be destroyed by prolonged heating with copper salts in alkaline solution. Furthermore, it is well known that other naturally occurring substances besides ascorbic acid may interfere in the method of Roe and Keuther if it is applied to crude materials with minute ascorbic acid concentration.

*Determination of Eosinophils in the Peripheral Blood.*—The animal was fasted fourteen hours, and a sample of blood was obtained from the femoral vein. The eosinophils were determined using the method of Randolph.[12]

## RESULTS

*Ascorbic Acid in Livers and Adrenal Glands.*—The average ascorbic acid content of the livers and adrenal glands in "control," "ascorbic acid deficient," and "scorbutic" groups of monkeys is presented in Table I. Arrangement of the data in these groups allows ready comparison between the

TABLE I. AVERAGE ASCORBIC ACID IN LIVERS AND ADRENAL GLANDS OF MONKEYS (MG. PER 100 GM., WET)

| CONDITION | NUMBER OF ANIMALS | LIVERS | ADRENALS |
|---|---|---|---|
| Controls, 50 mg. ascorbic acid per day | 4 | 12.9 | 49.9 |
| Ascorbic acid deficient | | | |
| 37 days | 1 | 1.2 | 4.9 |
| 82 days | 1 | 0.6 | — |
| Scorbutic | 10 | 0.8 | 3.4 |

tissue concentration of ascorbic acid and the urinary excretion of 17-ketosteroids in corresponding periods of the experiment. In the animals deprived of ascorbic acid there was a marked decline in both liver and adrenal ascorbic acid by the thirty-seventh day. Little further reduction occurred subsequently even with the advent and progression of scurvy. The significance of the residual trace of "apparent ascorbic acid" in scorbutic tissues has been discussed in the section on methods.

*Spontaneous Urinary Excretion of 17-ketosteroids.*—The average level of spontaneous urinary excretion of 17-ketosteroids in each group of monkeys is presented in Table II. These average figures were calculated from analyses of 34 specimens from 13 "control" animals, 5 specimens from an "adrenalectomized-control" animal, and 71 specimens from 18 monkeys deprived of ascorbic acid.

The spontaneous urinary excretion of 17-ketosteroids by the "adrenalectomized-control" monkey was significantly lower than that by either "control" animals or monkeys deprived of ascorbic acid. The presence of 17-ketosteroids in the urine of monkeys even after total adrenalectomy has been reported previously by Dorfman and associates.[13]

In monkeys deprived of ascorbic acid for twenty-eight days, the spontaneous urinary excretion of 17-ketosteroids was significantly lower than in "control" animals. This lower level of spontaneous excretion persisted until at or about the time of appearance of clinical signs of scurvy, when a suggestive rise toward the "control" level occurred. The number of serial determinations obtained during the onset and progression of scurvy is small and not amenable to statistical analysis. However, it is clear that in grossly scorbutic monkeys the spontaneous urinary excretion of 17-ketosteroids tended to equal or exceed that in "control" animals.

*Urinary Excretion of 17-ketosteroids During Stimulation with Corticotropin.*—The average maximum level of urinary excretion of 17-ketosteroids during stimulation with ACTH (Acthar Gel) in each group of monkeys is presented in Table III. These figures were calculated from analyses of 33 specimens from 11 "control" animals, 3 specimens from an "adrenalectomized-control" animal, and 99 specimens from 11 monkeys deprived of ascorbic acid.

The average maximum level of spontaneous urinary excretion of 17-ketosteroids in each group of monkeys is also presented in Table III. It is clear that in no group did this value equal or even approach the level observed during stimulation.

TABLE II. AVERAGE SPONTANEOUS URINARY EXCRETION OF 17-KETOSTEROIDS BY MONKEYS

| NO. OF ANIMALS | ANALYSES | CONDITION | 17-KETOSTEROIDS (MG./KILO./24 HR.) | S. D. |
|---|---|---|---|---|
| 13 | 34 | *Control* | 0.34 | 0.14 |
| | | *Days without ascorbic acid*: | | |
| 8 | 9 | 28-37 | 0.23 | 0.04 |
| 8 | 15 | 38-47 | 0.25 | 0.02 |
| 8 | 14 | 48-57 | 0.20 | 0.02 |
| 4 | 8 | 58-67 | 0.19 | 0.05 |
| 5 | 12 | 68-77 | 0.29 | 0.18 |
| | | (Average onset scurvy) | | |
| 8 | 16 | 78-90 | 0.21 | 0.08 |
| 3 | 3 | 90-99 | 0.38 | |
| 2 | 3 | 100-110 | 0.69 | |
| 1 | 1 | 120 | 0.38 | |
| 1 | 5 | *Adrenalectomized* | 0.13 | 0.02 |

There was no rise in the urinary excretion of 17-ketosteroids by the "adrenalectomized-control" monkey during stimulation. In none of the animals deprived of ascorbic acid did the rise in excretion during stimulation fall significantly below the value in the "control" monkeys. The last value recorded was obtained in a monkey with severe scurvy of about thirty days' duration and exhibits normal adrenocortical responsiveness, in spite of this severe and prolonged depletion of ascorbic acid.

*Eosinophils in the Peripheral Blood.*—There was a gradual decline in the level of eosinophils in the peripheral blood of the monkeys deprived of ascorbic acid and with the advent of scurvy the level was frequently zero, suggesting an increase in the activity of the adrenal cortex (Table IV). It has been reported that human beings with adrenal insufficiency may develop an eosinopenia during infection[14] or following large amounts of epinephrine.[15] Moreover, the "adrenalectomized-control" animal exhibited a marked depression of eosinophils during a wound infection. Because of these observations the eosinopenia in the "scorbutic" monkeys was considered inconclusive evidence of increased adrenocortical activity in scurvy.

TABLE III. AVERAGE MAXIMUM URINARY EXCRETION OF 17-KETOSTEROIDS BY MONKEYS

| | SPONTANEOUS EXCRETION | STIMULATED WITH ACTH (10 MG. GEL B.I.D.) | | |
|---|---|---|---|---|
| CONDITION | 17-KETOSTEROIDS (MG./KILO./24 HR.) | NO. OF ANIMALS | NO. OF ANALYSES | 17-KETOSTEROIDS (MG./KILO./24 HR.) |
| *Control* | 0.41 | 11 | 33 | 2.03 |
| *No ascorbic acid:* | | | | |
| 28-37 days | 0.24 | 8 | 23 | 1.99 |
| 38-47 days | 0.27 | 4 | 12 | 1.83 |
| 48-57 days | 0.21 | 8 | 23 | 2.01 |
| 58-67 days | 0.21 | 4 | 12 | 2.18 |
| 68-77 days | 0.31 | 2 | 6 | 1.50 |
| (Average onset scurvy) | | | | |
| 78-86 days | 0.20 | 7 | 14 | 2.19 |
| 87-94 days | 0.14 | 2 | 4 | 2.32 |
| 96-103 days | 0.35 | 3 | 4 | 3.17 |
| 112 days | 0.55 | 1 | 1 | 2.40 |
| *Adrenalectomized* | 0.14 | 1 | 3 | 0.14 |

TABLE IV. AVERAGE LEVEL OF EOSINOPHILS IN THE PERIPHERAL BLOOD OF MONKEYS

| CONDITION | NUMBER OF DETERMINATIONS | EOSINOPHILS PER CU. MM. |
|---|---|---|
| Controls | 27 | 186 |
| Ascorbic acid deficient | 14 | 116 |
| Scorbutic | 27 | 22 |

## SUMMARY AND DISCUSSION

Urinary 17-ketosteroids alone were measured without attention to the other adrenocortical hormones because of the relative simplicity of the method for the former and because the original intention was to compare responses to ACTH which stimulates production of both groups of substances. There may be discrepancies between the spontaneous excretion of 17-ketosteroids and other adrenocortical hormones in scurvy. Their excretion has often been found to vary similarly in other acute stressful situations.

The level of spontaneous urinary excretion of 17-ketosteroids by "ascorbic acid deficient" monkeys is lower than that by "control" animals. As scurvy appears and progresses, the level of spontaneous urinary excretion of 17-ketosteroids tends to equal or exceed the level of excretion in the "control" period. This rise in the level of spontaneous excretion is probably induced by circumstances in scurvy of a stressful nature. Even the most severely scorbutic monkeys responded to stimulation by ACTH with a rise in the level of excretion of 17-ketosteroids equal to that obtained from "control" animals. None of the monkeys in this experiment reached a phase of "adrenal exhaustion."

Since this study was initiated, reports have appeared in the literature concerning the urinary excretion of adrenocortical hormones by scorbutic guinea pigs. Clayton and Prunty demonstrated an initial decline in the spontaneous urinary excretion of 17-ketosteroids by guinea pigs deprived of ascorbic acid which was followed by a rise in the level of excretion above control levels with the onset of scurvy.[16] Nadel and Schneider reported a marked increase above control levels in the spontaneous urinary excretion of formaldehydegenic substances by the scorbutic guinea pigs.[17] The results of this investigation of the urinary excretion of 17-ketosteroids by scorbutic monkeys is in agreement with these studies. It seemed advantageous to evaluate the functional status of the adrenal cortex in another species and in periods of scurvy of longer duration than are tolerated by the guinea pig.

The information on the level of eosinophils is included because it emphasizes the fact that the level may be suppressed by factors other than the substances secreted by the adrenal cortex.

## CONCLUSIONS

This is a study of adrenocortical function in ascorbic acid deficiency and scurvy in which (a) the spontaneous urinary excretion of 17-ketosteroids and also the rise in excretion during stimulation with ACTH were measured; (b) the spontaneous urinary excretion of 17-ketosteroids by scorbutic monkeys tended to equal or exceed that of control animals; (c) the urinary excretion of 17-ketosteroids by scorbutic monkeys during stimulation with adreno-

corticotropin was as great as in control animals; (d) according to this index the function of the adrenal cortex in scorbutic monkeys is normal and is occasionally increased, presumably in response to stressful circumstances in scurvy; (e) the level of eosinophils in the blood will fall in response to factors other than adrenocortical hormones and cannot be depended upon as an index of adrenocortical function.

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# THE MEASUREMENT OF INTRAESOPHAGEAL PRESSURE AND ITS RELATIONSHIP TO INTRATHORACIC PRESSURE

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MEASUREMENT of intrathoracic pressure is of great importance in the understanding of the mechanics of pulmonary ventilation in health and disease. With no air moving in or out of the lungs, the relationship between intrathoracic pressure* ($P_T$), intrapulmonic pressure ($P_P$), and the elastic forces of the lung ($P_L$)† is

$$P_T = P_P - P_L \qquad (1)$$

If there is no obstruction in the airway, $P_P$ will equal atmospheric pressure or zero gauge pressure under static conditions.[1]

With air moving in or out of the lungs, $P_P$ will be negative on inspiration and positive on expiration, and will represent the pressure drop necessary to produce gas flow in the bronchopulmonary tree. In addition, a new increment of pressure ($P_V$) is necessary to overcome the viscous resistance of the tissues of the lung. This resistance must be overcome on both inspiration and expiration, and the resultant pressure increment will be negative on inspiration and positive on expiration. The equation therefore should be expanded to

$$P_T = P_P + P_V - P_L \qquad (2)$$

Other much smaller increments of pressure which could be added to the above equation are the acceleration forces for the lung, gas, and blood, and a correction of $P_L$ to include a pressure increment to allow for hysteresis.[2]

A study of the dynamic pressures in pulmonary and cardiac disease will cast much light on the processes involved. It therefore becomes of extreme importance to measure accurately the dynamic as well as the static intrathoracic pressure.

In the past, intrapleural pressure has been widely used as a measurement of intrathoracic pressure. This method has certain disadvantages in that there is some danger in the establishment of a pneumothorax in a normal human being and a larger danger in patients with pulmonary emphysema. Moreover, a large bore needle must be used if proper recording characteristics are to be obtained. For these reasons venous pressure[3] and intraesophageal pressure[4-7]

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*In this work the term *pressure* is defined as the difference between the absolute pressure in question and the prevailing atmospheric pressure. In the field of engineering this is called *gauge pressure.*

†This is, by convention, considered to be a positive quantity.

have been investigated as methods of measuring intrathoracic pressure. The purpose of this paper is to explore further the use of intraesophageal pressure as a method of measuring intrathoracic pressure.

Previous studies have strongly suggested that the esophagus reflects the intrathoracic pressures rather closely.[4-6] An excellent review of the world's literature, as well as much of the original work on the subject, appears in a doctorate thesis by Buytendijk.[4] In essence he points out that the esophagus is a thin, musculomembranous tube about 2 cm. in diameter and about 25 cm. in length which normally hangs in the thoracic cavity in a collapsed state connected to the other structures of the mediastinum by varying amounts of loose areolar tissue and several sets of small muscles. At the upper and lower ends there are functional sphincters. The pressure variations in the esophagus are

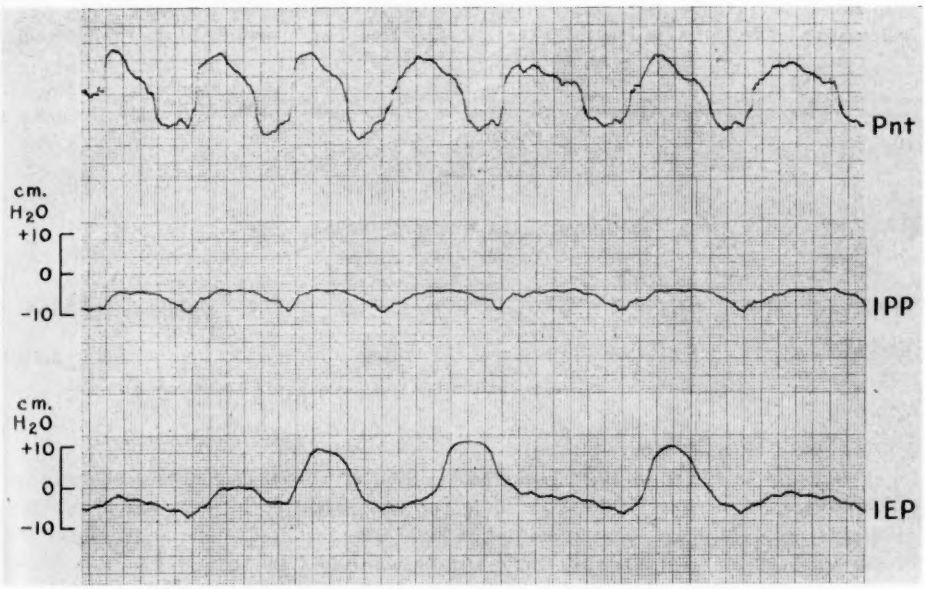


Fig. 1.—The simultaneous recording of the velocity of the respiratory stream with a pneumotachygraph (*Pnt*), intrapleural pressure (*IPP*) and intraesophageal pressure (*IEP*). This tracing was taken shortly after swallowing the balloon, and demonstrates waves of easily recognizable esophageal spasm. (Paper speed: 10 mm. per second.)

of intrinsic and extrinsic origin. The extrinsic pressures are those of the intrathoracic cavity. Buytendijk has described the various intrinsic disturbances of the pressure in detail. These intrinsic pressures are essentially of two types: first, localized spasms or tonus changes which may occasionally spread and become generalized; second, peristaltic waves. These intrinsic pressure changes fortunately are usually easily recognizable (Fig. 1). Triggering mechanisms for these changes in tone are described by Buytendijk, and should be avoided to obtain a record which is free of intrinsic pressure disturbances. The most important triggering mechanisms are swallowing, pharyngeal irritation, esophageal irritation or distention, extreme expiration or inspiration, and psychic disturbances such as anxiety. Consequently, a small, nonirritating

intraesophageal tube should be used if satisfactory intraesophageal pressure tracings are to be obtained. The patient should be comfortable and familiarized with the objective and procedure of the experiment.

## METHOD

The intraesophageal pressures were recorded with a special type of intraesophageal recording balloon. Numerous intraesophageal recording devices were evaluated. The one chosen was a siliconized polyethylene tube on the end of which was a thin latex balloon (Fig. 2). Pressure acting upon the balloon was transmitted by the tube to a strain gauge transducer* of appropriate characteristics.

The balloon† was long and sausage-shaped, with a wall thickness of 0.005 cm., a diameter of 1 cm., and a length of 18 cubic centimeters. It was made very thin in order to interfere as little as possible with the recording fidelity of this system. The sole purpose of the balloon was to prevent mucus from entering the recording tube. The balloon was made 18 cm. in length so that pressure recording apertures in the tubing would

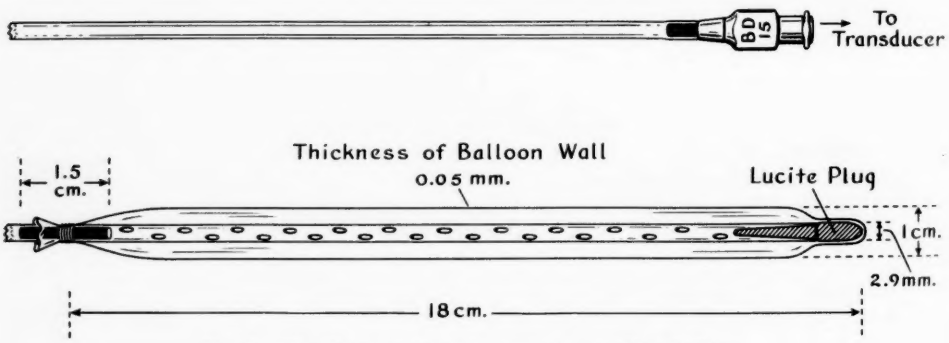


Fig. 2.—The intraesophageal pressure recording balloon.

lie throughout the length of the intrathoracic portion of the esophagus, thus allowing the pressure recording system to be exposed to the most negative intraesophageal pressure existing at any given moment. Therefore, if there were localized spasm at the upper end of the esophagus, a good recording could still be obtained from the lower portion of the esophagus. The balloon contained only 2 to 4 ml. of gas during the studies, and hence was in a relatively collapsed state.

The recording tube leading from the balloon to the pressure transducer was polyethylene tubing‡ with a length of 80 cm., an inside diameter of 0.177 cm., and an outside diameter of 0.280 cm. A No. 15 needle was introduced into the end of the tubing as an adaptor for attachment to the strain gauge. The tube was inserted through the nose to a distance of 42 cm. between the external nares and the tip of the tube. This placed the proximal end of the balloon slightly below the suprasternal notch, and the distal end of the balloon just above the cardia.

The diameter of the tubing was experimentally chosen to give good recording characteristics for the system. Since the tube was to be passed through the nose, the smallest possible tube was desired. Frequency response curves were determined with tubing of various diameters attached to the pressure transducer. Fig. 3 shows such a family of

*Model P6-2D-250 2 pounds per square inch. Statham Laboratories, Berkeley, Calif.

†The balloons were manufactured especially for this purpose through the courtesy of the American Anode Co., Akron 8, Ohio.

‡Obtained from the Clay Adams Co., Inc., 141 E. 25th St., New York, N. Y., Cat. No. PE250.

frequency response curves obtained by varying the tube diameters. The smallest diameter which would allow a resonant frequency well above the fastest components to be measured was chosen. This tube was then optimally damped by experimentally varying the length of the needle attaching the tubing to the pressure transducer. The addition of the balloon to the tube selected did not change the frequency response curve (Fig. 3). The damped natural frequency of this system was about 28 cycles per second. The calculated natural frequency was 34 cycles per second using air as a recording medium.

The natural frequency of a simple recording system is given by the equation,

$$\omega_n = \sqrt{\frac{K}{M}} \qquad (3)$$

where $\omega_n$ is the natural frequency expressed as angular velocity, M is the vibrating mass, and K is the spring constant or restorative force per unit displacement.

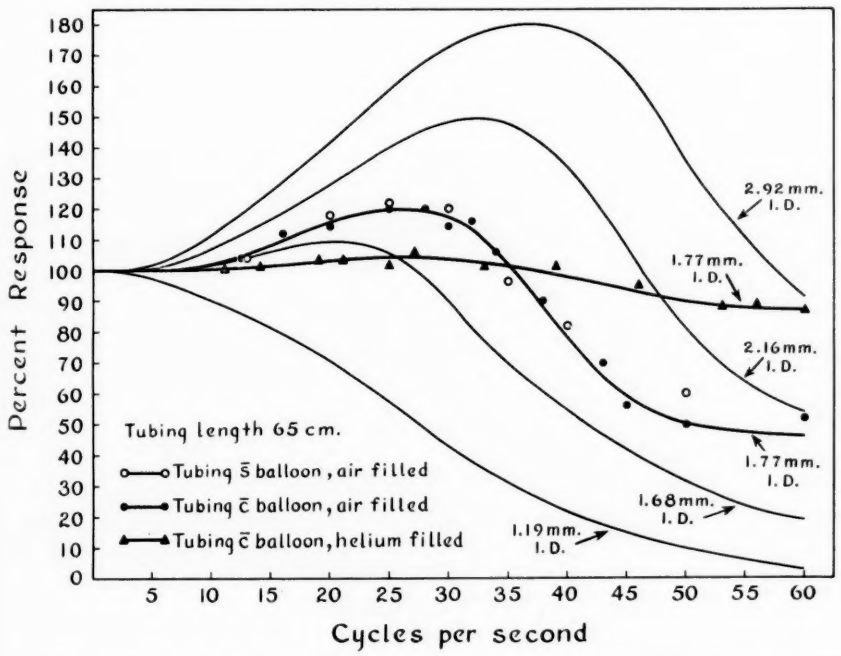
Percent Response
Cycles per second
Tubing length 65 cm.
Tubing S balloon, air filled
Tubing C balloon, air filled
Tubing C balloon, helium filled
2.92 mm. I. D.
1.77 mm. I. D.
2.16 mm. I. D.
1.77 mm. I. D.
1.68 mm. I. D.
1.19 mm. I. D.

Fig. 3.—Frequency response curves for various tubing diameters. These curves were obtained with a sinusoidal pressure pulse generator. For simplicity the pure frequency response curve for the galvanometers was omitted. Its course followed that of the helium-filled balloon out to about 40 cycles per second, at which point it began to drop off more rapidly and intersected the 60 cycles per second line at about 70 per cent.

A recording system such as described in this paper is a compound one, and is made up of three components, each of which has a natural frequency of its own. These components are the direct writing galvanometers, the elements of the strain gauge transducer, and the recording medium in the transmitting tubing and transducer cavity. These components act in series as three links in a chain. The pressure wave is passed from one component to the next. The natural frequency of the above type of system will be that of the component of the system which has the lowest natural frequency.

The strain gauge transducer which was used in this study had a natural frequency of 600 cycles per second.* This was far above the frequencies occurring in physiological

*This figure was supplied by the Statham Laboratories, Bulletin No. 3.1 Section A, Statham scientific instruments, Low pressure transducers.

pressure recording, and consequently its effect on the system was ignored. The galvanometers of the amplifying recorder,* when properly adjusted, had a natural frequency of about 30 cycles per second as measured in this laboratory, but, because of a feed-back circuit, they had a useful range extending to about 40 cycles per second. The experimentally observed natural frequency of the air-filled pressure transmitting system was 28 cycles per second. This natural frequency, being the lowest of the three components of the system, was the natural frequency of the entire system. This is an adequate natural frequency to avoid significant amplitude distortion in most physiologic pressure recordings.

Phase lag distortion is a slightly more serious problem in a compound recording system such as the one described in this study. The phase lag, $\Theta$, increases in each vibratory system with increase in frequency of vibration according to equation 4,

$$\tan \Theta = \frac{\omega \cdot c}{K - M.\omega^2} \qquad (4)$$

where $\omega$ is the angular velocity and C is the damping coefficient.[8] The over-all phase lag of the entire system is equal to the sum of the phase lags in each vibratory component.

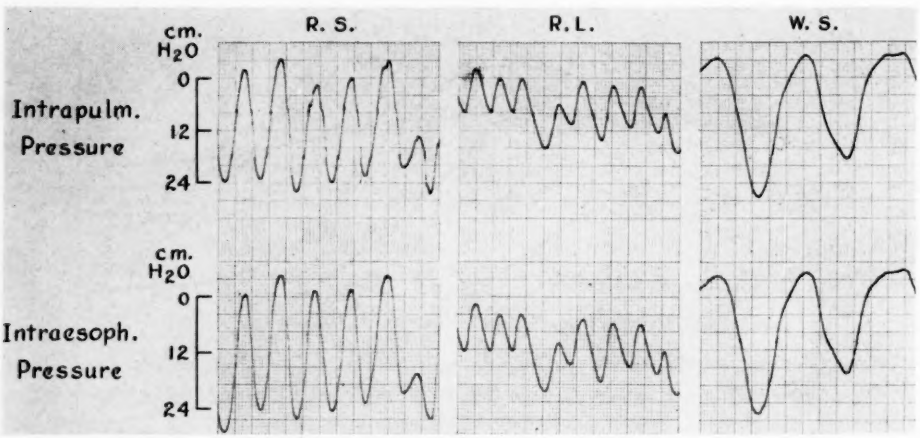


Fig. 4.—Simultaneous recorded intraesophageal and intrapulmonary pressures. (Paper speed: 25 mm. per second.)

It therefore becomes apparent that amplitude distortion, as well as phase distortion, may be improved by making K as large as possible and M as small as possible in each vibratory component. The K in equations 3 and 4 cannot be altered, as the compressibility of different gases in the transducer cavity is essentially the same, and the size of the cavity cannot be changed. The mass of the gas in the pressure transmitting tubing, however, may be easily reduced, thus decreasing M. The density of helium is about one-eighth that of air. Since the natural frequency varies inversely with the square root of the vibrating mass (equation 3), substitution of helium for air should afford a natural frequency almost three times as large and a corresponding decrease in phase lag as shown by equation 4.

The result of the substitution of helium for air may be seen in Fig. 3. The frequency response of the recording system when filled with helium was limited only by the damping off of the frequency response of the recording galvanometers.

*Sanborn Poly Viso amplifying recorder, Sanborn Company, Cambridge, Mass.

## RESULTS

In order to evaluate the validity of intraesophageal pressure as a measure of intrathoracic pressure, the relationship between intraesophageal and intrathoracic pressure was studied in two ways. The first method consisted of simultaneously measuring the intraesophageal and intrapulmonic pressures on two equally calibrated strain gauges. The electrical output of these strain gauges was fed into the four-channel amplifying recorder. With the intraesophageal balloon in place and attached to one of the strain gauges, the subject was asked to exert pressure against the second gauge with an open glottis. As mentioned previously, intrathoracic pressure varies with intrapulmonic pressure according to equation 1, provided the volume of the lungs does not change during the period under consideration. Rearranging equation 1 we obtain

$$P_L = P_P - P_T \qquad (5)$$

$P_L$ is a function of the volume of the lung. Under the circumstances of the experiment the volume of the lung and thus $P_L$ remains constant except for

TABLE I. COMPARISON OF INTRAESOPHAGEAL (IE) AND INTRAPULMONARY (IP) PRESSURE CHANGES*

| SUBJECT | CHANGE IN IP ($CM.H_2O$) | CHANGE IN IE ($CM.H_2O$) | $\frac{\text{CHANGE IN IE}}{\text{CHANGE IN IP}} \times 100$ |
|---|---|---|---|
| R. S. | 26.0 | 27.3 | 105 |
| | 24.4 | 22.8 | 93 |
| | 27.6 | 26.6 | 96 |
| | 30.4 | 28.4 | 93 |
| | 24.4 | 25.2 | 103 |
| | 22.4 | 23.8 | 106 |
| | 24.0 | 24.2 | 101 |
| | 22.4 | 22.8 | 102 |
| | 26.4 | 25.2 | 95 |
| | 24.4 | 23.5 | 96 |
| | 6.0† | 3.9 | 65 |
| | 12.8† | 9.1 | 71 |
| R. L. | 10.0 | 10.3 | 103 |
| | 10.0 | 10.3 | 103 |
| | 7.6 | 7.7 | 101 |
| | 7.6 | 7.7 | 101 |
| | 7.6 | 7.7 | 101 |
| | 16.0 | 16.8 | 105 |
| | 10.0 | 10.3 | 103 |
| | 4.4 | 4.6 | 105 |
| | 9.6 | 10.0 | 104 |
| | 13.6 | 13.8 | 101 |
| | 12.4 | 12.6 | 102 |
| | 9.6 | 9.6 | 100 |
| | 9.2 | 9.2 | 100 |
| | 10.4 | 10.7 | 103 |
| | 4.4† | 5.0 | 114 |
| | 8.8† | 9.6 | 109 |
| W. S. | 31.6 | 29.8 | 94 |
| | 32.0 | 30.6 | 96 |
| | 23.2 | 21.8 | 94 |
| | 24.0 | 22.6 | 94 |
| Mean | | | 98.2 per cent |

*The figure read from top to bottom in this table are the serial measurements of corresponding simultaneous intrapulmonary and intraesophageal pressure changes from left to right in Fig. 4.

†During this portion of the tracing the glottis was closing.

insignificant changes associated with compression of the gas. $P_P$ and $P_T$ vary directly with each other to maintain a constant difference, $P_L$. Consequently, if the changes in intraesophageal pressure equal those in intrapulmonic pressure, we can assume that intraesophageal pressure is an accurate means of measuring *changes* in intrathoracic pressure.

Study of the relationship of the intraesophageal to the intrathoracic pressure by this method is illustrated in Fig. 4. This illustration shows the simultaneous recording of intrapulmonic pressure and intraesophageal pressure in three normal men. The measurements of the simultaneous pressure fluctuation

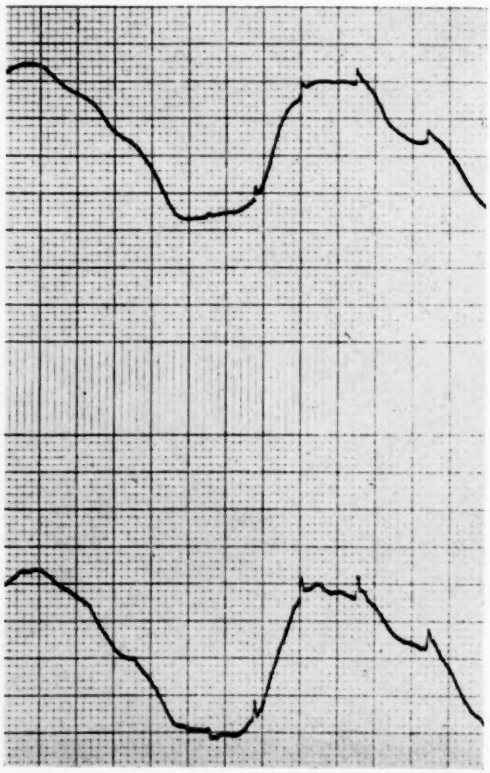

Fig. 5.—Simultaneously recorded intraesophageal and intrapulmonary pressures demonstrating phase relationships. (Paper speed: 25 mm. per second.)

in Fig. 4 are shown in Table I. Analysis revealed that the amplitude of the intrapulmonic pressure fluctuations was reflected accurately in the intraesophageal pressure tracing provided the glottis remained open during the respiratory efforts of the subjects and there was no esophageal spasm. There was no phase lag. This was well shown by striking the chest of a patient with a percussion hammer and recording the resulting pressure spikes (Fig. 5).

The second method of study consisted of simultaneously recorded pressures from the esophagus and pleural cavity of a young man with a spontaneous pneumothorax. Only a very small amount of air remained in the

pleural cavity at the time of the experiment. A 15-gauge needle (4 cm. in length) was introduced into the small remaining rim of the pneumothorax, and a 20 cm. piece of plastic tubing (3 mm. inside diameter) was used to attach the needle to one of two nearly equally calibrated strain gauges. The second strain gauge simultaneously recorded the intraesophageal pressure. A pneumotachygraph, modified from the one described by Lilly,[9] was used to record the velocity of the respiratory stream. The electrical output of the pneumotachygraph and of the two gauges was fed into three channels of a recording amplifier. The results are illustrated in Fig. 6. It can be seen that the relative change in intrapleural pressure was quantitatively reflected by intraesophageal

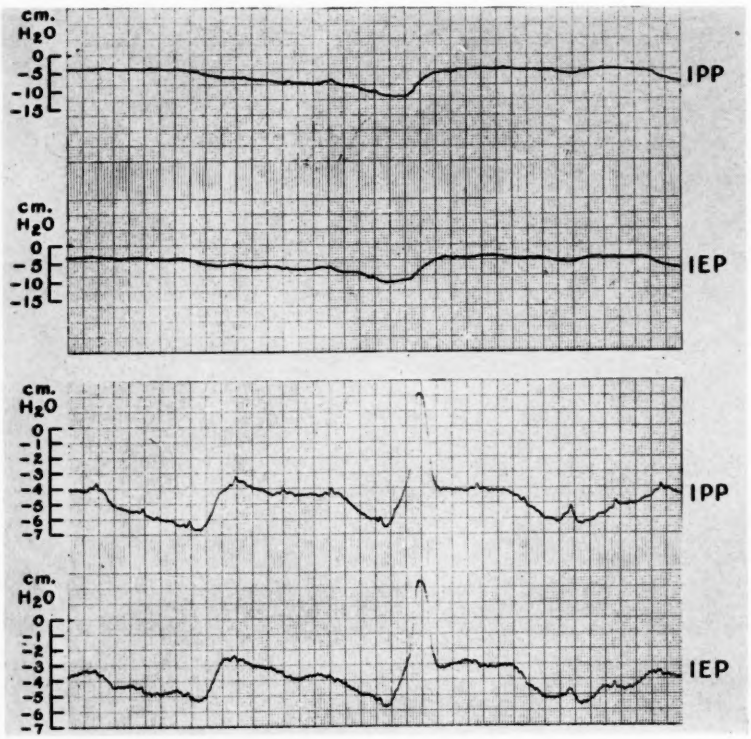


Fig. 6.—Simultaneously recorded intrapleural (*IPP*) and intraesophageal (*IEP*) pressures. The lower tracing was taken at five times greater amplification than the upper tracing. (Paper speed: 25 mm. per second.)

pressure when the changes were of low frequency. However, when the frequency of the pressure change was increased by panting (Fig. 7), it became apparent that the intrapleural pressure lagged behind the intraesophageal pressure. A phase lag of 0.02 second and a drop in amplitude of about 25 per cent occurred in the intrapleural pressure changes during panting. Therefore, the helium-filled intraesophageal pressure recording balloon was more accurate than the intrapleural method for determining rapid changes in intrathoracic pressure. The intrapleural pressure was consistently slightly more negative than the intraesophageal pressure, as can be seen in Fig. 6.

## DISCUSSION

Three basic methods of determining the intrathoracic pressure have been explored in the past. These are measurement of the intrapleural pressure after establishment of a pneumothorax, the peripheral venous pressure, and the esophageal pressure. All of these methods have certain disadvantages.

The peripheral venous pressure method[3] lends itself only to measurement of approximate changes in static intrathoracic pressure and can be performed only in trained subjects. It is valueless as a method of measuring dynamic intrathoracic pressure. This seriously limits its practical use.

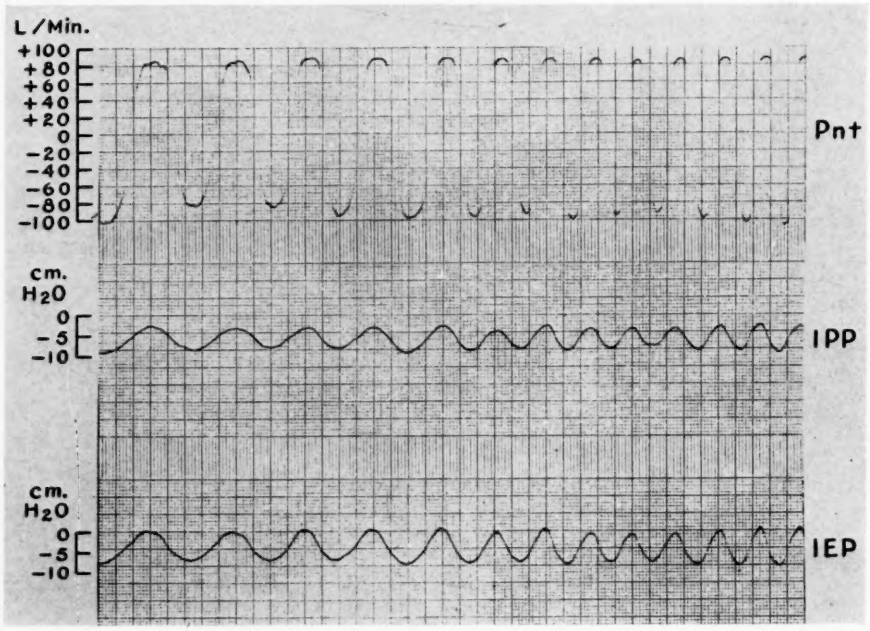


Fig. 7.—Simultaneous pneumotachygraph (*PNT*), intrapleural pressure (*IPP*), and intraesophageal pressure (*IEP*) tracings at the maximum respiratory frequency of the subject. This shows the damping off of the intrapleural pressure recording with rapidly changing pressures. (Paper speed: 50 mm. per second.)

The method of measurement of the intrapleural pressure has the distinct disadvantage of risk of air embolism, and puncture of a bulla or bleb with the possibility of a tension pneumothorax. In order to have any reasonable recording fidelity one must use a trocar of at least 2 mm. inside diameter to avoid excessive damping. Moreover, air is of necessity the recording medium rather than helium. The tip of the trocar may run afoul of the lung and will always have a layer of fluid, fibrin, and other matter inside of it. Consequently, recording fidelity is difficult or impossible to obtain even in a carefully designed system for intrapleural pressure measurement. The only advantage of intrapleural pressure is that it affords an accurate measurement of static intrathoracic gauge pressure provided truly static conditions are achieved.

The use of intraesophageal pressure as a measure of intrathoracic pressure has only one significant disadvantage. Subtle changes in esophageal tone which may not be apparent on the tracing invalidate the use of intraesophageal pressure as an accurate measure of intrathoracic gauge pressure. This disadvantage is of little importance in the study of the mechanics of ventilation since pressure *changes* are of much greater significance than actual gauge pressures. It appears from the theoretical considerations and the experimental results presented in this paper that measurement of the static and dynamic intrathoracic pressure changes may be most safely and accurately accomplished by measurement of the intraesophageal pressures.

## SUMMARY

An intraesophageal pressure recording balloon was described. The importance of the use of helium in any pressure recording system in which a gas is used as a pressure transmitting medium was demonstrated.

It was shown that intraesophageal pressure changes are quantitative reflections of intrathoracic pressure *changes* when the esophagus is relaxed. This was demonstrated by comparing the change in intraesophageal pressure with the change in intrapulmonary pressure, and by direct measurement of the intrapleural pressure simultaneously with intraesophageal pressure.

It is concluded that the use of intraesophageal pressure is a safe and accurate method of measuring changes in intrathoracic pressure.

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# THE ELASTIC PROPERTIES OF THE LUNG IN NORMAL MEN AND IN PATIENTS WITH CHRONIC PULMONARY EMPHYSEMA

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IT HAS been known for many years that the lung possesses elastic properties. This retractive force coupled with the weight and elastic forces of the expanded chest is the force that produces the normal expiration.[1] Moreover, a balance between the passive forces of the chest wall and the elastic forces of the lung determines the functional residual volume of the lung.[2] One of the characteristic findings in patients with pulmonary emphysema is an increase in functional residual volume, and it is generally believed that this increase is related to a change in the elastic properties of the lung. It therefore becomes of considerable interest to quantitate the elastic forces of the lung in normal subjects and in patients with pulmonary emphysema.

## METHOD

In defining the elastic properties of the lung, it is necessary to relate the retractive force of the lung ($P_L$) to the volume of the lung. This relationship was studied in the following manner. The subject breathed from a recording spirometer through a three-way respiratory valve (Fig. 1, *B*). Between the mouthpiece and the valve a pressure tap led to the negative side of a differential strain gauge. The positive side of the strain gauge was attached to the intraesophageal recording balloon which has been demonstrated in the preceding paper[3] to transmit changes in intrathoracic pressure ($P_T$). The pressure tap between the subject and the respiratory valve transmitted intrapulmonic pressure ($P_P$) when there was no respiratory flow and an open glottis. The strain gauge recorded the difference between these two pressures. This difference in pressure was caused by the retractive force of the lung ($P_L$)[1] as illustrated by the equation,

$$P_L = P_P - P_T$$

The function of the respiratory valve was to produce momentary interruption of the respiratory flow in order that pressures could be obtained under static conditions. A pneumotachygraph (Fig. 1, *A*) was inserted in the inspiratory arm of the spirometer circuit to indicate zero velocity of air flow.

In the performance of the procedure the esophageal pressure recording balloon was introduced through the nose after initial topical anesthetization. The subject breathed oxygen from the spirometer in a comfortable sitting position (Fig. 1). After a brief record of normal ventilation had been obtained, the subject was instructed to inspire deeply, during which time the respiratory valve was intermittently closed. A steplike pressure tracing was recorded (Fig. 2). Plateaus (Fig. 2, *SP*) occurred during the period of closure of the valve, and represented the retractive force of the lung ($P_L$). The changes in lung

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volume corresponding to these pressures were related to a sloping straight line drawn through the lower points of the tidal excursions in the spirometer tracing (functional residual volume). After a series of inspirations had been obtained, the subject was asked to expire completely and the valve was again closed intermittently (Fig. 3).

The increments of pressure were plotted against the corresponding lung volume changes on linear graph paper. This relationship was expressed in absolute terms by determining the functional residual volume by the open circuit method[4] and the mean $P_L$ at the functional residual volume (Figs. 4 and 5). The graphs thus obtained express a relationship between the change in the volume of the lung and the resulting change in $P_L$. This pressure volume curve is analogous to a stress-strain diagram in engineering.

In the analysis of the pressure tracings, artifacts were excluded. These artifacts arose essentially from two sources: closure of the glottis (Fig. 3), and increases in esophageal tone (Fig. 2). When a relatively horizontal pressure plateau with clearly defined corners was obtained, these artifacts were considered to be absent.

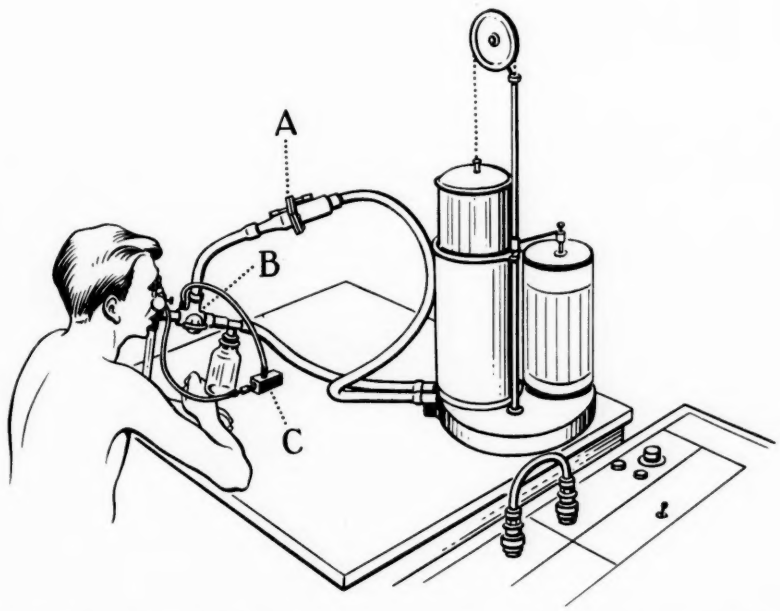


Fig. 1.—Diagram of apparatus. *A*, pneumotachygraph; *B*, three-way respiratory valve; *C*, 2 p.s.i. differential strain gauge, the negative side (upper attachment) of which is recording intra-oral pressure and the positive side (attachment toward subject) is recording from the intraesophageal balloon.

## RESULTS

Ten normal men and ten patients with chronic pulmonary emphysema were studied by this method. The diagnosis of pulmonary emphysema was made on the basis of symptoms, physical signs, roentgenogram of the chest, and an increase in the ratio of residual volume to total lung volume (Table I). Representative pressure-volume curves are shown in Figs. 4 and 5. The important points to note are: (1) the changes in the lung compartments in the emphysematous patient as compared to the normal man; (2) the preservation

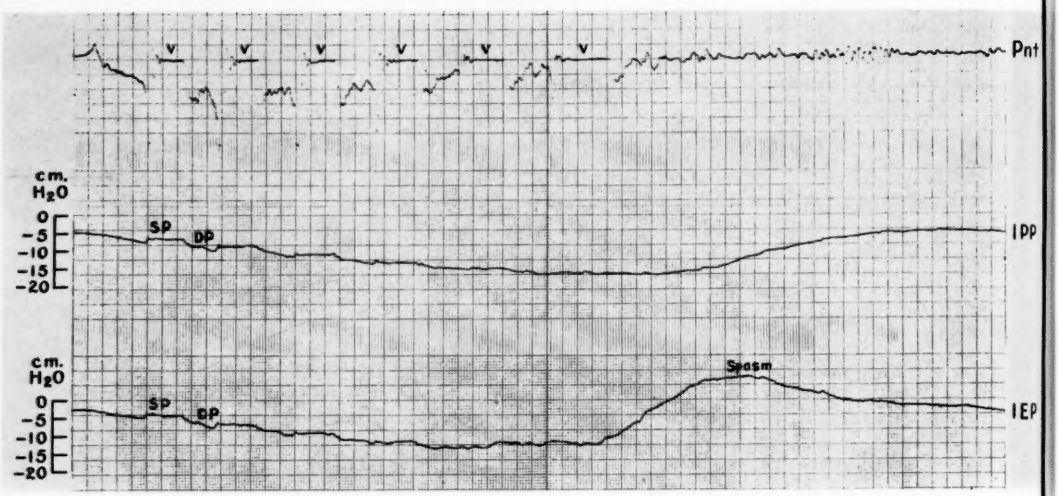


Fig. 2.—Interrupted inspiration. Pneumotachygraph (*Pnt*) recording at top of illustration shows an interrupted deep inspiration. The horizontal part of the tracing under each "*V*" indicates zero respiratory flow during the closure of the respiratory valve. Note the progressive decrease in the simultaneously recorded intraesophageal (*IEP*) and intrapleural pressures (*IPP*) as the inspiration progresses until a wave of increased esophageal tone occurs at the maximum inspiratory effort. *SP* = static pressure plateau; *DP* = dynamic pressure.

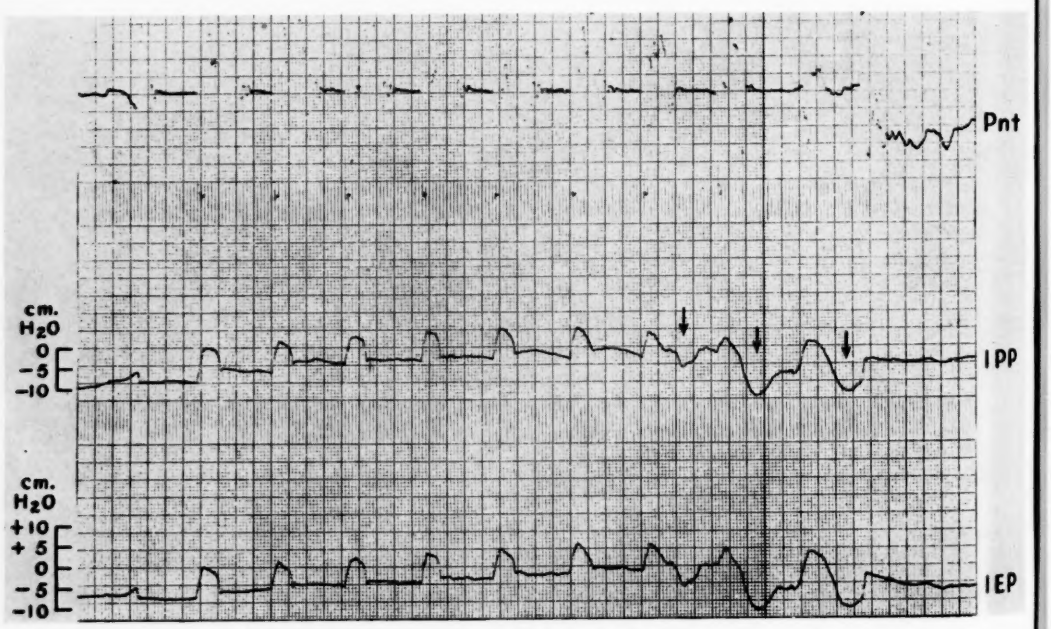


Fig. 3.—Same as Fig. 2 except that a forced expiration is shown. Note that dynamic pressure is in a positive direction. Toward the end of the expiratory effort the subject closed his glottis. This produced a negative intrapulmonic pressure and thus a more negative intrathoracic pressure.

of a functional relationship between pressure and volume in the emphysematous patient; and (3) the curvilinear shape of the pressure-volume relationship.

Although the relationship between pressure and volume is curvilinear, it may be considered approximately linear in the region of the tidal volume. The slope of this line has been described in previous studies[5] as the change in pressure per 100 c.c. change in lung volume. This method of analysis has been applied to the pressure-volume curves of the normal men and patients with pulmonary emphysema (Table I). The mean change in pressure per 100 c.c.

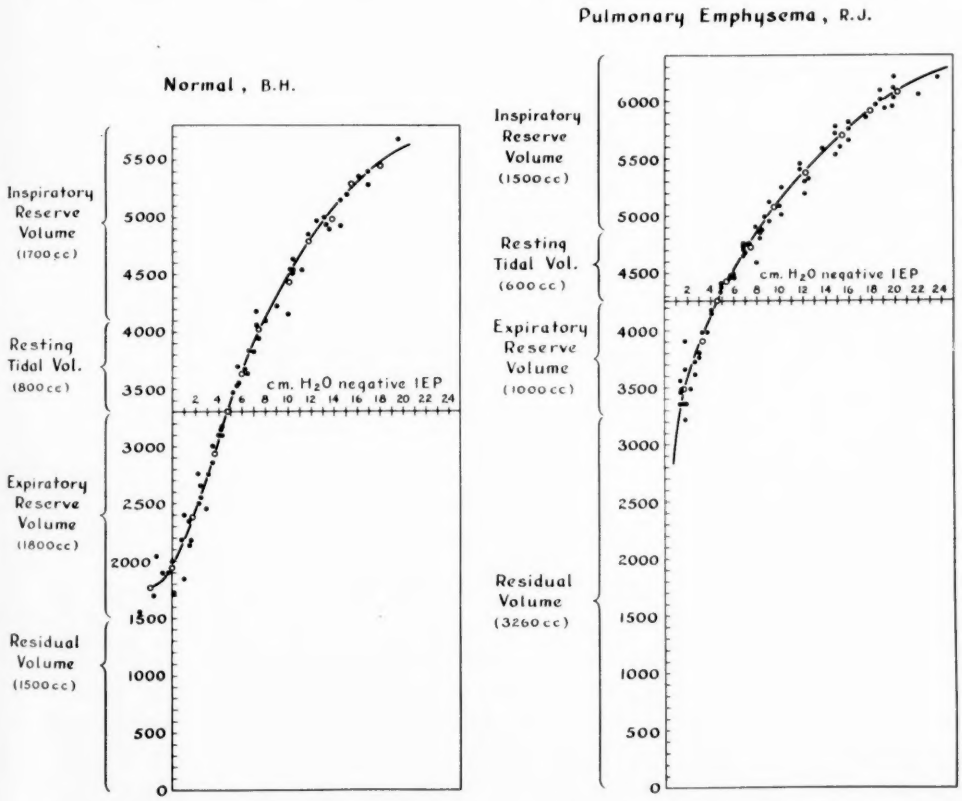


Fig. 4.—The pressure volume diagram of the lung of a normal subject as compared to that of a patient with emphysema. The curves are related to atmospheric pressure and total lung volume. Open circles are the mean points. Solid circles are actual points.

change in lung volume in normal men was 0.43 cm. of water (standard deviation 0.096). The corresponding value for the patients with pulmonary emphysema was 0.56 cm. of water (standard deviation 0.14). The difference between the two mean values was statistically significant ($p = 0.02$). It may be noted that the variation between individuals was greater in the group of patients with emphysema than in the normal men. The difference between intraesophageal pressure and atmospheric pressure at the functional residual volume is also given in Table I. The pressures were less negative in the patients with pulmonary

TABLE I. INTRAESOPHAGEAL PRESSURE IN NORMAL SUBJECTS AND PATIENTS WITH PULMONARY EMPHYSEMA

| SUBJECT | AGE | TOTAL LUNG VOLUME | VITAL CAPACITY | RATIO OF RESIDUAL VOLUME TO TOTAL LUNG VOLUME | CHANGE IN INTRAESOPHAGEAL PRESSURE IN CM. $H_2O$ PER 100 C.C. CHANGE IN LUNG VOLUME | INTRAESOPHAGEAL GAUGE PRESSURE AT FUNCTIONAL RESIDUAL VOLUME |
|---|---|---|---|---|---|---|
| | | | *Normal subjects* | | | |
| R. E. | 39 | 6280 | 4300 | 32 | 0.61 | 9.6 |
| R. M. | 27 | 6060 | 4950 | 18 | 0.40 | 6.8 |
| W. S. | 33 | 6970 | 4840 | 31 | 0.35 | 6.4 |
| B. H. | 35 | 5800 | 4300 | 26 | 0.35 | 4.8 |
| R. S. | 31 | 5590 | 4450 | 20 | 0.36 | 2.2 |
| R. P. | 34 | 7700 | 5850 | 24 | 0.32 | 4.0 |
| D. F. | 27 | 6950 | 5770 | 17 | 0.40 | 8.8 |
| F. M. | 33 | 4930 | 3250 | 34 | 0.50 | 5.2 |
| F. McD. | 29 | 6300 | 4780 | 24 | 0.55 | 8.0 |
| E. R. | 29 | 5930 | 4400 | 26 | 0.41 | 4.4 |
| Mean | 31.7 | 6251 | 4689 | 25.1 | 0.43 | 6.02 |
| Standard deviation | | | | | 0.096 | 2.33 |
| | | | *Patients with pulmonary emphysema* | | | |
| C. N. | 69 | 7280 | 2770 | 62 | 0.64 | 4.8 |
| V. F. | 60 | 6030 | 2900 | 52 | 0.45 | 5.4 |
| S. D. | 60 | 5090 | 2510 | 52 | 0.60 | 3.1 |
| F. W. | 58 | 7080 | 2710 | 62 | 0.75 | 2.7 |
| H. T. | 61 | 6460 | 2040 | 68 | 0.71 | 4.7 |
| P. M. | 52 | 5490 | 2420 | 56 | 0.50 | 1.9 |
| R. J. | 65 | 6310 | 3050 | 52 | 0.59 | 4.4 |
| T. C. | 67 | 5480 | 2010 | 63 | 0.58 | 2.7 |
| K. M. | 58 | 6950 | 2940 | 58 | 0.25 | 3.1 |
| S. B. | 39 | 5330 | 2860 | 46 | 0.53 | 3.7 |
| Mean | 58.9 | 6150 | 2621 | 57.1 | 0.56 | 3.65 |
| Standard deviation | | | | | 0.142 | 1.13 |

emphysema than in the normal men. The difference between the means was statistically significant ($p = 0.01$).

A young man with a small spontaneous pneumothorax was also studied. There was no evidence of underlying pulmonary disease. The method was the same as that previously described, and in addition simultaneous intrapleural pressures were recorded differentially with the intrapulmonary pressure. The pressure recordings from this subject are illustrated in Figs. 2 and 3. Fig. 5 illustrates the pressure-volume curve derived from the intraesophageal pressures and that derived from the intrapleural pressures. Note: (1) the slight sigmoid character of the curve obtained from the intrapleural pressure, (2) the parallel course of the two curves above the functional residual volume, and (3) the close agreement even in gauge pressures.

## DISCUSSION

The elastic forces of the lung are of great importance in the mechanics of ventilation. The amount of air in the lungs at the end of a normal expiration (the functional residual volume) is determined by a balance between the elastic forces of the lung and the passive forces of the chest wall. Several previous

investigators have found the relationship between the volume of the normal lung and the elastic force of the lung to be linear.[6-8] In the present study the relationship was found to be curvilinear, although the deviation from linearity was not great in the region of the tidal volume. Analysis of the pressure required to produce a change of 100 c.c. in the volume of the lung gave a result which was similar to that obtained by Otis, Rahn, and Fenn[5] using venous pressure

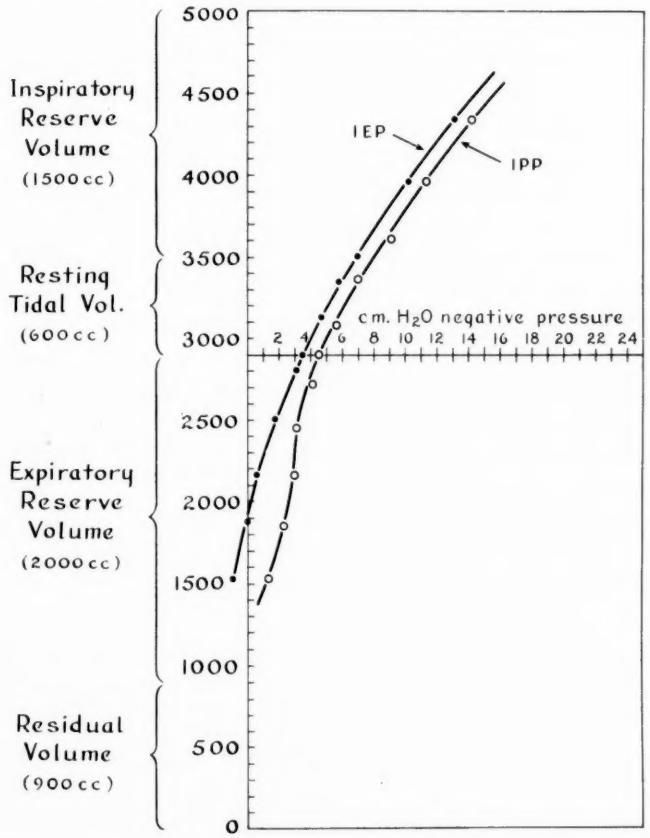


Fig. 5.—Pressure volume diagrams of the lung of a patient with a small spontaneous pneumothorax. The curve IEP is constructed from intraesophageal pressure measurements and the curve IPP from intrapleural pressure measurements. Each point represents the mean of of about six measurements.

measurements and Buytendijk[8] using intraesophageal pressure but smaller than that obtained by Christie and McIntosh,[7] Paine,[9] and Dayman[10] using intrapleural pressure measurements.

It is generally accepted that the basic defect in chronic pulmonary emphysema is a loss of the normal retractive force of the lung. As a result, the balance between the retractive force of the lung and the passive forces of the chest wall is altered, and the functional residual volume is increased. From a study of intrapleural pressure and tidal volume, Christie[11] concluded that the lung in pulmonary emphysema had an almost complete loss of elastic forces.

This conclusion was based on the lack of a linear relationship between change in volume of the lung and change in intrapleural pressure. Unfortunately, in these studies there was no clear differentiation between static and kinetic forces. As pointed out by Neergaard and Wirz,[12] measurements of intrapleural pressure must be made at zero velocity of air flow if these measurements are to be used as an expression of the elastic forces of the lung. Christie was unable to make his measurements at zero velocity because of the nature of his recording system (water manometer). Paine,[9] in a study of intrapleural pressure in pulmonary emphysema, also failed to differentiate clearly between static and kinetic forces. He used a unit of distensibility which was defined as the maximum difference in intrapleural pressure that occurred in a normal breath divided by the tidal volume. It is apparent that this unit will depend on the velocity of air flow and the viscous properties as well as on the elastic forces of the lung. This confusion of static and kinetic pressures is most unfortunate in the study of the elastic properties of the lung in pulmonary emphysema because in this disease the kinetic pressures are large, presumably because of obstruction to air flow. Paine also attempted to measure pressures at zero velocity, but was unable to obtain reproducible results, probably because of phase lag in his recording system. For this reason the results obtained by Christie and by Paine bear little relation to the results obtained by measuring pressures at zero velocity of air flow.

In the present study the lung was found to have retained its retractive force in pulmonary emphysema, and the relationship between this force and change in volume of the lungs with inspiration was also found to be approximately linear in the region of the tidal volume. The change in intraesophageal pressure for each 100 c.c. increase in volume of the lungs was slightly greater in the group of patients with emphysema as compared with the normal group. There was a considerable overlapping of values between the two groups. These results correspond well with the values obtained by Buytendijk[8] using intraesophageal pressure and with the result on one patient with emphysema studied by Neergaard and Wirz.[12]

The chief difference between the normal men and the patients with pulmonary emphysema lay in the size of the functional residual volume. As mentioned previously, the functional residual volume is determined by a balance between the passive forces of the chest wall and the retractive force of the lung. It appears from the present study and from previous studies that the retractive force of the lung is altered in pulmonary emphysema. Fig. 4 clearly illustrates that the whole lung elasticity curve is moved upward in patients with pulmonary emphysema as compared with normal subjects. This means that the lung has retained some retractive force but that the functional relationship of this retractive force to changes in lung distention has been altered. Whether this is the sole cause of the increase in functional residual volume or whether in addition there is an alteration in the passive forces of the chest wall cannot be stated from the present study.

SUMMARY

Using intraesophageal pressure as a measure of intrathoracic pressure, the retractive force of the lung was studied in normal subjects and in patients with pulmonary emphysema.

Curves were constructed relating the change in retractive force of the lung to the change in lung distention. These curves were curvilinear, but were approximately linear in the region of the tidal volume. The change in retractive force of the lung for a 100 ml. change in lung volume in this portion of the curve was greater in the patients with pulmonary emphysema than in the normal men.

In patients with pulmonary emphysema, the relationship of the retractive force of the lung to the degree of lung distention was greatly modified. This alteration in the elastic forces of the lung contributes to the increase in functional residual volume in this disease.

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# IMPAIRMENT OF ANTIMICROBIAL DEFENSES FOLLOWING TOTAL BODY IRRADIATION OF MICE

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A PRETERMINAL bacteremia is quite consistently demonstrable in animals dying about eight to fifteen days after systemic x-irradiation in the $LD_{50}$ range.[1-3] The organisms have in most instances been identified as members of the normal bowel flora, and it has been presumed that they gain access to the tissues as a result of injury to intestinal epithelium. Yet that injury is maximal very soon after irradiation, whereas bacteremia occurs several days later.

Of perhaps greater significance than the entry of bacteria into the blood stream is the apparent inability of normal removal mechanisms to function adequately under these circumstances. In normal animals and man, bacteria from the respiratory or intestinal tract sporadically gain access to the blood stream, but such transient bacteremias are of some consequence only when an associated abnormality, such as a previously damaged heart valve, leads to the focal establishment of infection. In the absence of such abnormality microorganisms are promptly filtered out of the circulation by reticuloendothelial elements, particularly in spleen, liver, bone marrow, and lung, and are subsequently destroyed by cellular and humoral defense mechanisms.

In irradiated animals, these mechanisms appear to be deficient. Organisms entering the blood stream multiply in unrestrained fashion, resulting in a bacteremia and toxemia which are responsible, at least in part, for mortality. The series of experiments described in this paper were designed to elucidate the nature of the defect induced by irradiation in the normal antimicrobial defense mechanisms.

## MATERIAL AND METHODS

*Mice.*—Homozygous strain $C_{57}$ black mice, originally obtained from Dr. L. C. Strong of Yale University School of Medicine, and inbred by a brother-to-sister mating in the laboratories of the Department of Radiology, Stanford University School of Medicine during the past three years, were used in all experiments. The animals were of both sexes and $33 \pm 3$ days of age (14 to 18 Gm.) at the time of irradiation and/or infection. Littermates were distributed at random among all experimental groups. The colony is free of spontaneous infection, and spontaneous deaths have not exceeded 0.1 per cent.

*Irradiation.*—Physical factors were 120 KVP, 9 Ma, 0.25 mm. Cu + 1.00 mm. Al added filter, 30 cm. mouse-target distance, 32 r per minute. Animals were irradiated in

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shallow, perforated lucite boxes with a wall thickness of 2 millimeters. Exposures were measured in air with a Victoreen r-meter placed at the position of the lucite containers. A minimum of scattering material was present in the treatment field.

*Infection.*—A standard subacute infection was induced in the manner previously described[4] by a single intramuscular injection of 100 $LD_{50}$ (0.1 ml. of a $10^{-4}$ dilution of an eighteen-hour culture in blood broth) of a beta hemolytic streptococcus (C 203) into the left thigh. In most experiments animals were infected one to three days after irradiation, except as otherwise specified below. Two types of end points were studied: the number of daily deaths, and the rate of spread of infection in serially sacrificed animals. Groups of thirty to forty animals were observed closely for the development of illness, and deaths were recorded daily. In parallel groups similarly treated, three to five mice per group were sacri-

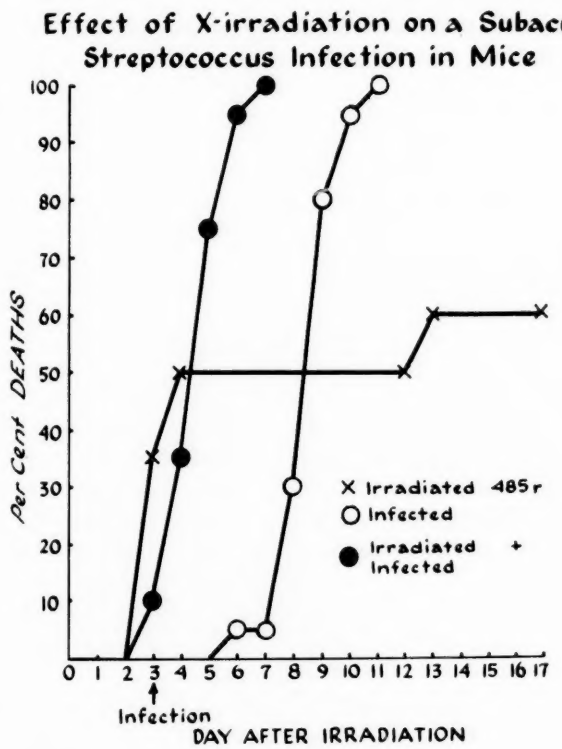


Fig. 1.

ficed daily, and semiquantitative cultures were made on blood agar plates from the injected thigh muscle, regional lymph node, liver, spleen, lung, bone marrow, and heart blood. The method of culture was the same as that previously described.[4] Total white blood cell counts and differential counts were performed on representative members of some groups.

*Treatment.*—In some experiments groups of mice were treated with commercial crystalline penicillin G or with aureomycin hydrochloride (Lot *7-6116*) made available by Dr. Stanton S. Hardy, Lederle Laboratories. The drugs were administered daily for three days by subcutaneous injection of a volume of 0.2 milliliter. Solutions for injection were prepared freshly each day. The daily dose of penicillin G was 36 to 120 μg, of aureomycin 50 to 250 μg. Parallel groups were observed for daily number of deaths and for rate of dissemination of bacteria as described above.

## RESULTS

I. *Rate of Spread of Infection in Irradiated Mice.*—It has been reported previously that the subacute streptococcal infection used in these experiments exhibits a gradual orderly progression from the site of injection.[4] Bacteria reach the regional lymph nodes during the first twenty-four hours, but are retained there for hours or even days. Occasional organisms that reach the blood stream on the second or third day after inoculation are promptly filtered out by the spleen, liver, and bone marrow, delaying the onset of overwhelming bacteremia and death until five to seven days after infection. This rather leisurely progression of the infection in unirradiated animals is in striking contrast to the course of events in mice subjected to total body irradiation with 485 r, forty-eight hours prior to inoculation with the microorganisms (Table I). In the latter animals, progress of the infection is rapid and overwhelming. The organisms rapidly pass the radiation-damaged lymphatic barrier and appear in large numbers in the blood stream in twenty-four to forty-eight hours. Under these circumstances, the filtration mechanisms appear unable to remove bacteria from the circulation effectively, and the animals succumb rapidly with overwhelming bacteremia.

This sequence of events is mirrored in the cumulative death curves shown in Fig. 1. Death is accelerated by approximately four days in the irradiated, infected group as compared with the infected controls. It is of interest, however, that the two curves are virtually parallel. This suggests that the bacteremia, once developed, is equally rapidly lethal in both groups, and that they differ only in the rate of development of bacteremia. Some of the earliest deaths in the irradiated group were attributable to other radiation damage, since irradiated uninfected controls also exhibited deaths during this phase, but deaths in excess of the mortality suffered by the irradiated controls were clearly due to acceleration of the spread of infection in the virtually complete absence of antimicrobial defenses.

II. *Duration of Impairment of Antimicrobial Defenses.*—Groups of thirty mice each received a single total-body dose of 450 r and were then inoculated

TABLE I. EFFECT OF IRRADIATION ON THE DISSEMINATION OF A SUBACUTE STREPTOCOCCAL INFECTION IN MICE

| IRRADIATION | TISSUES CULTURED | DAYS AFTER INFECTION | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 |
| None | Muscle | + | ++++ | +++ | ++++ | ++++ | +++ |
| | Lymph node | 0 | 0 | ++ | ++++ | +++ | +++ |
| | Liver and spleen | 0 | + | + | +++ | +++ | +++ |
| | Lung | 0 | + | 0 | + | + | ++ |
| | Heart blood | 0 | + | + | ++ | +++ | +++ |
| 485 r two days before infection | Muscle | +++ | ++++ | ++++ | | | |
| | Lymph node | 0 | +++ | ++ | | | |
| | Liver and spleen | 0 | +++ | ++++ | All mice dead | | |
| | Lung | 0 | ++ | ++ | | | |
| | Heart blood | 0 | ++++ | ++++ | | | |

Thirty mice per group; 4 mice autopsied each day.

0, No bacteria cultured; +, moderate growth in 1 or 2 mice cultured; ++, moderate growth in 3 or 4 mice cultured; +++, heavy growth in 2 or 3 mice cultured; ++++, heavy growth in all mice cultured.

intramuscularly with the standard infection at one, three, seven, eleven, and fifteen days after irradiation, respectively. Littermate control groups were infected at the same times. All animals were observed closely for evidence of illness, and deaths were recorded daily. Average day of death and standard error for each group are recorded in Fig. 2, in which the data for the control groups have been pooled, since variation among the groups was found to be minimal. Impairment of defenses is apparent within twenty-four hours after systemic irradiation and becomes maximal between the third and seventh days, with a gradual return to normal by the fifteenth day after irradiation.

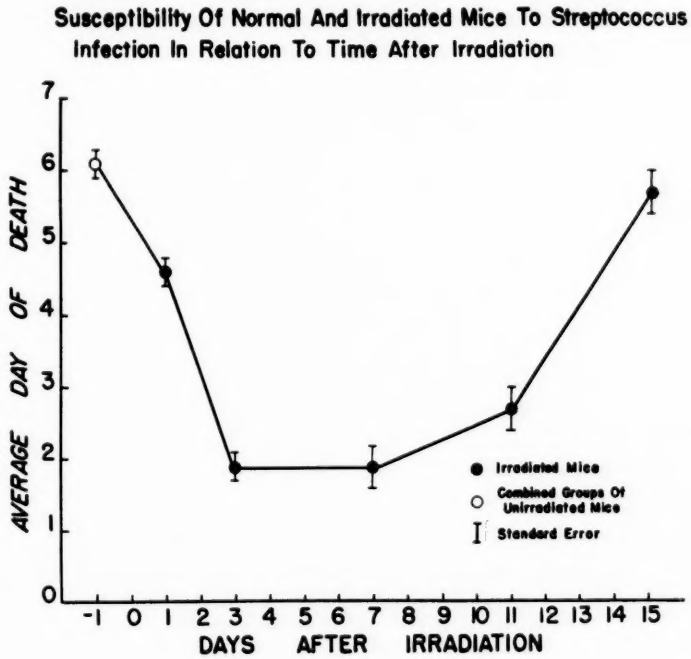


Fig. 2.

III. *The Effect of Treatment with Penicillin.*—In unirradiated animals, treatment with penicillin started twenty-four hours after infection promptly and completely eliminated all microorganisms. Even at the site of injection streptococci could be recovered only for twenty-four to forty-eight hours.

In mice not given chemotherapy, the polymorphonuclear response was quite intense on the third and fourth days after infection (Table II). There was no such leukocytic response in the penicillin-treated animals. In the irradiated-infected mice treated with penicillin, cumulative mortality was not significantly greater than that in irradiated uninfected controls (Fig. 3). Thus penicillin was able to prevent deaths due to infection. However, microorganisms were not completely eliminated from the tissues at the site of inoculation. Histologic studies revealed liquefaction necrosis and relative avascularity in the injected area suggesting that it may have been relatively inaccessible to the drug; and

there was no local accumulation of polymorphonuclear leukocytes, in contrast to the extensive cellular exudate in infected controls. It is assumed that these factors combine to permit local persistence of microorganisms. After penicillin administration was discontinued in the irradiated animals, occasional streptococci reached the circulation, but there was no recrudescence of susceptibility to their dissemination and no late deaths from bacteremia under these circumstances.

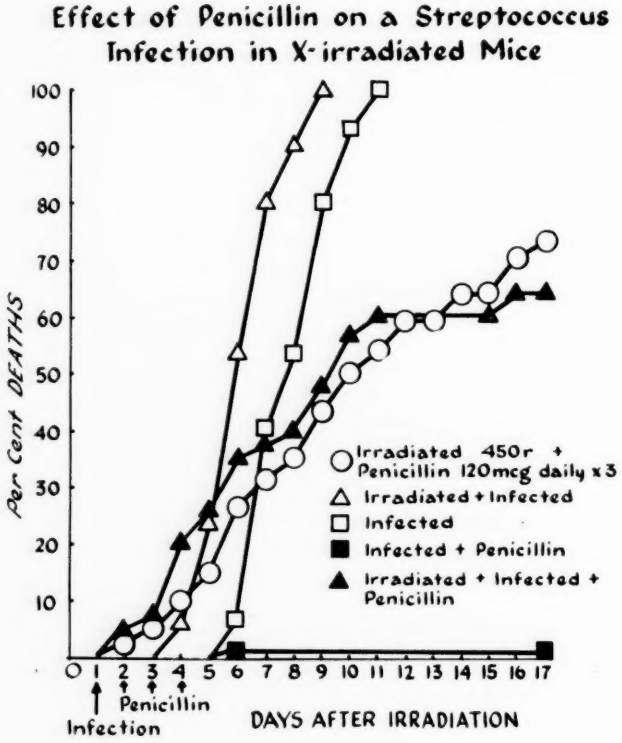


Fig. 3.

It appears that penicillin in large doses is capable of controlling microbial proliferation sufficiently to prevent deaths from infection superimposed on radiation injury even in the virtual absence of polymorphonuclear leukocyte phagocytic activity. This is consistent with the demonstrated effectiveness of several antibiotics in the treatment of spontaneous infections in irradiated animals of various species.[5-7] On the assumption that the effectiveness of penicillin in the present study was attributable to its known bactericidal action on the streptococcus employed,[4] it might be inferred that a bacteriostatic agent should be less effective in irradiated infected animals. To test this point, aureomycin was administered.

IV. *Treatment with Aureomycin.*—The dose of aureomycin used was sufficient to protect unirradiated-infected animals, in which there was presumably

sufficient phagocytic response to assist the bacteriostatic action of the drug. Fig. 4 summarizes the effect of aureomycin on irradiated-infected animals. It will be seen that, although mortality in the treated group was reduced below that of irradiated-infected animals, it remains significantly greater than that of irradiated-uninfected controls, suggesting that protection by aureomycin at this dose level is less complete than that provided by penicillin.

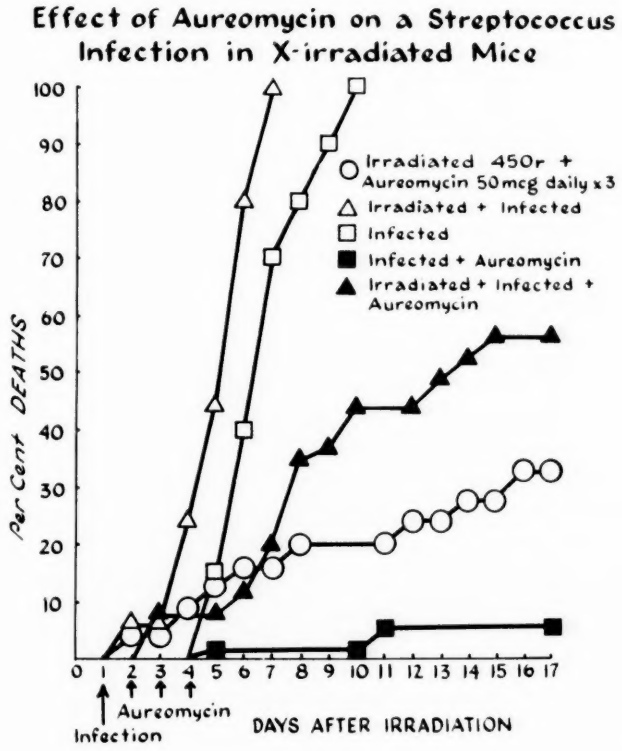


Fig. 4.

V. *Effect of Spleen-Shielding.*—In a single preliminary experiment, two groups of ten mice each received a single systemic dose of 450 r with their spleens exteriorized and shielded in a lead container as described by Jacobson and associates.[8] One of the groups was then inoculated with the standard infection twenty-four hours later. Spleen shielding appeared to afford no protection against death from bacteremia in irradiated $C_{57}$ black mice under these circumstances. This observation provoked another experiment in which it was observed that spleen shielding is much less effective in the protection of strain $C_{57}$ black mice against postirradiation mortality than it is in similarly treated strain A mice.[9] Therefore the lack of protection by spleen shielding in the present experiment against the spread of lethal infection in irradiated $C_{57}$ black mice is probably a genetically limited observation requiring additional studies in other strains and species.

TABLE II. EFFECT OF PENICILLIN ON SPREAD OF STREPTOCOCCAL INFECTION AND ON LEUKOCYTE COUNT IN INTACT AND IRRADIATED MICE

| TREATMENT | TISSUES STUDIED | DAYS AFTER INJECTION | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | 5 | 6 |
| Infection alone | Muscle | | ++ | ++++ | ++++ | ++++ | ++++ | ++++ |
| | Lymph node | | 0 | ++ | + | + | ++++ | ++++ |
| | Liver, spleen, lung | | 0 | 0 | ++ | ++ | +++ | ++++ |
| | Heart blood | | 0 | + | ++ | + | +++ | ++++ |
| | PMN's ave./cu. mm* | | 4000 | 4200 | 6800 | 8300 | 3800 | 3530 |
| | No. of deaths | | 0 | 0 | 0 | 0 | 2 | 3 |
| Infection and Penicillin 200 units/day ×3 subcutaneously | Muscle | | + | 0 | 0 | 0 | 0 | 0 |
| | Lymph node | | 0 | 0 | 0 | 0 | 0 | 0 |
| | Liver, spleen, lung | | 0 | 0 | 0 | 0 | 0 | 0 |
| | Heart blood | | 0 | 0 | 0 | 0 | 0 | 0 |
| | PMN's ave./cu. mm. | | 4000 | 3700 | 3000 | 3200 | 3300 | 2750 |
| | No. of deaths | | 0 | 0 | 0 | 0 | 0 | 0 |
| Irradiation, 450 r 1 day before infection, and penicillin 200 units/day × 3 subcutaneously | Muscle | | ++++ | ++++ | +++ | +++ | +++ | ++++ |
| | Lymph node | | 0 | 0 | 0 | 0 | + | 0 |
| | Liver, spleen, lung | | 0 | 0 | 0 | 0 | + | + |
| | Heart blood | | 0 | 0 | 0 | 0 | + | + |
| | PMN's ave./cu. mm. | 1500 | 450 | 350 | 60 | 60 | 50 | 65 |
| | No. of deaths | | 1 | 4 | 1 | 0 | 0 | 0 |

*Absolute polymorphonuclear leukocyte counts on tail vein blood of 2 to 4 mice per group daily; 20 mice.

Twenty mice per group; 3 to 4 mice autopsied daily.

0, No bacteria cultured; +, moderate growth in 1 or 2 mice cultured; ++, moderate growth in 3 or 4 mice cultured; +++, heavy growth in 2 or 3 mice cultured; ++++, heavy growth in all mice cultured.

## DISCUSSION

A standard subacute streptococcal infection has been shown to spread from the site of inoculation and to establish a fatal bacteremia far more rapidly in mice exposed to a single total-body irradiation in the $LD_{50}$ range than in non-irradiated controls. At this dose level, damage to the host defenses against bacterial invasion and proliferation becomes apparent within twenty-four hours, and reaches a maximum between three and seven days, with a gradual return to normal levels by fifteen days after irradiation. That this time sequence is a function of radiation dose, however, is suggested by the work of Shechmeister, Bond, and Swift,[10] who found that susceptibility of mice to air-borne streptococcus zooepidemicus after a single sublethal exposure to 350 r was measurably increased three days after irradiation, rising to a maximum at approximately fifteen days, and returning to normal levels between thirty and thirty-five days after irradiation. Although the time sequence of diminished resistance after $LD_{50}$ doses in the present work roughly parallels the cycle of destruction and regeneration of lymphoid and myeloid tissues and of circulating white blood cells, the more prolonged, though milder, impairment of defenses noted by Shechmeister and associates could not be correlated with histologic or hematologic changes induced by sublethal doses of irradiation.[10]

The effects noted in the present experiments would appear to involve at least two systems: (1) the defense mechanisms normally concerned with limiting spread of microorganisms from the site of entry into and beyond the regional lymph nodes, and (2) those mechanisms normally concerned with filtration and destruction of bacteria which gain access to the circulating blood. There is evidence that both types of mechanisms are injured by total body

irradiation. That the peripheral tissue defenses may be differentially damaged by *local* irradiation is suggested by the work of several investigators (cited in the review by Taliaferro and Taliaferro[11]), who noted earlier necrosis and larger lesions containing a greater number of organisms after local treatment of streptococcal and staphylococcal infections. Townsend and Campbell[12] emphasized the paucity of cellular infiltration into experimental inflammations in mice and rabbits exposed to massive whole-body irradiation, and attributed this inhibition of tissue response to the profound coexistent leukopenia induced by radiation. The streptococcus employed in the present experiments normally excites a polymorphonuclear leukocyte reaction at the site of inoculation, and there was a striking absence of this cellular infiltrate in the irradiated mice, which exhibited only focal liquefaction necrosis in the injected muscle. Braude[13] has stressed the importance of the polymorphonuclear leukocyte in primary phagocytosis and transport of Brucella from the site of intraperitoneal inoculation through the blood stream to the reticuloendothelial cells of the liver and spleen. It seems clear that the destruction of these cells is a most important factor in the diminished local resistance of irradiated animals to the spread of bacterial infection.

There is a striking parallelism between the effects of irradiation on bacterial infection and those of cortisone on susceptibility of rabbits to the establishment of a lethal streptococcal infection, recently reported by Mogabgab and Thomas.[14] Although defense mechanisms were very similarly impaired in the cortisone-treated animals, polymorphonuclear leukopenia did not occur until streptococcal bacteremia was well established, and was not thought to contribute to the observed decrease in resistance. Dougherty and Schneebeli[15] suggested that the relative scarcity of infiltrating leukocytes at the site of experimental inflammations in cortisone-treated animals may be due to inhibition of the humoral stimuli which normally attract such cells, and designated this as the antiphlogistic effect of cortisone. The possibility that irradiation, in additon to the massive destruction of leukocytes, may also impair the stimuli which draw these cells from the blood stream to the site of local infection deserves careful experimental study.

However, neither the leukopenia nor the lack of local cellular infiltrate explain the failure of systemically irradiated mice to dispose of microorganisms after their appearance in the circulating blood. As early as 1935, Chrom[1] injected living Salmonella intravenously into mice and noted that, whereas normal animals were able to clear the blood of bacteria within twelve to fifteen hours, the heart blood of mice subjected to a whole body dose of 450 r did not become sterile within twenty-four to seventy-two hours. When he shielded the liver and spleen during irradiation, the blood became sterile within sixteen hours. He concluded that the fixed reticuloendothelial cells of the liver and spleen in particular were injured by systemic irradiation and that their filtering action upon circulating bacteria was impaired. Doubt has recently been cast upon this interpretation by Barrow, Tullis, and Chambers,[16] who found that 1,000 r total body irradiation had no consistent effect on phagocytosis as measured by the 50 per cent disappearance time of col-

loidal radiogold injected intravenously into rabbits. Moreover, Brecher and associates[17] noted no deficiency in the ability of irradiated mice to phagocytose nuclear debris or carbon particles. The question arises whether the phagocytosis of intravenously injected inert particulate matter is a proper measure of the capacity of the macrophage system to take up and destroy virulent bacteria. This variation in response may also be a function of radiation dose; it was recently noted by Taplin and co-workers[18] that the phagocytic removal of intravenously injected prodigiosin by the macrophage system of rabbits was depressed after doses of 800 r, but not after 300 r. Additional quantitative studies of the influence of irradiation upon the latter process are needed, with particular emphasis upon variations from one species to another. That the leukopenia following irradiation is probably not responsible for the impaired clearance of bacteria from the circulating blood is suggested by the work of Kerby and Martin.[19] They found that splanchnic removal of intravenously injected bacteria was not disturbed in the presence of severe benzol-induced leukopenia in rabbits.

It is well known that irradiation inhibits the formation of antibodies,[20] but it seems unlikely that specific antibodies play a significant role in combating the subacute streptococcal infection used in these experiments, in which death normally occurs within five to seven days. Although there is a "superinfection" immunity while active infection persists, animals quickly cured by chemotherapy fail to exhibit immunity to subsequent reinfection.[21] It is therefore doubtful that effects of irradiation on antibody formation were involved in the impairment of antimicrobial defenses noted in these experiments.

It is of interest that large doses of penicillin were effective in eliminating deaths due to streptococcal infection in these irradiated mice, even in the presence of a profound leukopenia and without the aid of the normal host defense mechanisms. This is consistent with the known bactericidal action of penicillin in vitro upon this organism. Penicillin in large doses was also effective in the treatment of cortisone-induced streptococcal infection in rabbits.[14] The somewhat lesser effectiveness of aureomycin in the present experiments is probably a function of dosage. At lower doses it is likely that some organisms persist whose complete elimination requires the active participation of the host defense mechanisms.

## SUMMARY

A standard subacute streptococcal infection spread more rapidly in $C_{57}$ black mice subjected to single total-body x-ray exposures in the $LD_{50}$ range than in nonirradiated controls. Cumulative mortality curves in the two groups were parallel but the average survival time was significantly reduced by irradiation. Using the average day of death as a criterion, impairment of resistance was apparent within twenty-four hours after irradiation at this dose level, increased to a maximum between three and seven days after irradiation, and gradually returned to normal by fifteen days after treatment.

Penicillin in large doses started within twenty-four hours after infection was effective in eliminating deaths due to bacteremia in the irradiated mice,

despite the presence of a profound leukopenia. Aureomycin was somewhat less effective at the dose levels studied.

In a preliminary experiment, spleen shielding failed to protect $C_{57}$ black mice against the profound impairment of antimicrobial defenses caused by systemic irradiation. It has since been shown that this response may be genetically conditioned, in that spleen-shielding of mice of this strain confers relatively little protection against the lethal effect of radiation in contrast to the striking protection noted in other strains.

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# SERUM ACETYLCHOLINESTERASE

## Its Close Correlation with Serum Albumin, and Its Limited Usefulness as a Test of Liver Function

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A DIRECT correlation between nonspecific acetylcholinesterase activity and albumin concentration in human pathologic sera was first reported by Faber[1] in 1943, and was subsequently confirmed by Kunkel and Ward[2] and by Levine and Hoyt.[3] Faber also noted that this positive correlation was consistently found in multiple serum specimens obtained from most patients. Exceptions appeared to coincide with rapidly changing clinical status, the serum albumin concentration reflecting the clinical course more promptly than the enzyme activity. Acetylcholinesterase activity was determined by these investigators with relatively complex manometric techniques.

Since the publication in 1949 by Michel[4] of a simple and satisfactory potentiometric method for determining serum acetylcholinesterase activity, there has been considerable interest in its potential clinical usefulness. The enzyme determination has been recommended as a test of liver function, both as an aid in the differential diagnosis of hepatobiliary disease,[5, 6] and as a sensitive indicator of changing liver function during the course of parenchymal liver disease.[6, 7] A positive correlation between the serum albumin concentration and the acetylcholinesterase activity, as measured by the Michel method or a slight modification of it, has recently been questioned.[5, 8]

The present data demonstrate a difference of statistical significance between the mean serum acetylcholinesterase activity of patients with advanced portal cirrhosis and the mean enzyme activity in the sera of patients with acute viral hepatitis, obstructive jaundice, or intra-abdominal carcinoma without cachexia. Clinical considerations which do not submit to statistical analysis, however, indicate that the measurement of serum acetylcholinesterase activity does not provide information of diagnostic importance in hepatobiliary disease which is not readily available from established laboratory procedures.

A positive correlation of statistical significance between the enzyme activity and the albumin concentration in pathologic sera is also presented. Contrary to Faber's report,[1] a temporal parallelism between the two determinations was demonstrated in almost every case where serial observations

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were obtained, including patients undergoing rapid changes in clinical status. The data indicate that the measurement of serum acetylcholinesterase activity is useful for following the course of patients with advanced portal cirrhosis or with severe viral hepatitis in the acute phase. In this series, serial determination of the serum enzyme activity often provided a prognostic guide of greater sensitivity than the laboratory tests now in common use for this purpose.

## METHODS

Determinations of acetylcholinesterase and albumin were performed in duplicate upon fasting serum separated from the clot within two hours of venipuncture. Serum samples were frequently frozen until several specimens had accumulated.*

Acetylcholinesterase activity was measured in serum and in ascitic fluid by the method of Michel.[4]† The correction factors proposed by Michel for nonenzymatic hydrolysis, and for decreasing enzyme activity with increasing acidity, were used after preliminary investigation had confirmed their accuracy. Results were easily reproducible, and are reported in *units of pH change per hour.*

Serum albumin was separated from other serum proteins by precipitation of the globulins with a sodium sulfate-sulfite reagent‡ described by Reinhold and co-workers.[9] The mean albumin concentration in abnormal sera, as determined by biuret reaction following this method of separation, has been found by Reinhold and associates[9] to be 0.27 Gm. per cent lower than that determined by electrophoresis. In this laboratory, the nitrogen of the albumin filtrate was determined by micro-Kjeldahl digestion, employing the mercury catalyst of Hiller, Plazin, and Van Slyke,[10] distillation of ammonia from the Kirk apparatus[11] into 2 per cent boric acid solution, and semimicrotitration of the distillate with standard sulfuric acid.

The statistical significance of the difference in the mean serum acetylcholinesterase activity between the various groups of patients was determined by Student's "t" test.[12] The significance of the correlation coefficients between acetylcholinesterase activity and albumin concentration was found by the use of Fisher's "t".[12]

## RESULTS

Acetylcholinesterase activity was determined one or more times in the sera of 40 normal individuals and 157 patients with a variety of diseases seen at the King County Hospital. The findings from initial determinations are summarized in Table I.

The normal mean value of 0.86 units in this series is slightly lower than the mean enzyme activity of 0.94 units found in normal sera by Vorhaus and associates[6] and that of 1.08 units recently reported by Mann and co-workers.[13] The probable explanation lies in the higher percentage of females in our normal group; Butt and associates[14] first observed the statistically lower activity of acetylcholinesterase in the sera of normal females as compared with males.

*Serum Acetylcholinesterase and the Differential Diagnosis of Hepatobiliary Disease.*—Each group of pathologic sera had a significantly lower acetylcholinesterase activity than the group of normal sera (Table I). Sera from patients with advanced portal cirrhosis had a mean enzyme activity which was significantly lower than that found in the sera of patients with acute viral hepa-

*The observation of Vorhaus and associates[6] that serum acetylcholinesterase activity is preserved in frozen samples for many months has been confirmed in this laboratory. The enzyme activity also remains unaltered for as long as twenty-four hours at room temperature, but decreases markedly if kept at 5° C. for one week.

†The acetylcholine chloride used as the substrate was kindly provided by Merck and Company, Inc., Rahway, N. J.

‡Sodium sulfate, 1.47, sodium sulfite 0.56 moles per liter.

titis, benign obstructive jaundice, or intra-abdominal carcinoma without cachexia. No significant difference in mean enzyme activity existed between the latter three groups. These findings are at variance with the report by Alcalde[5] that the serum acetylcholinesterase activity is helpful in differentiating neoplastic obstructive jaundice from benign obstructive jaundice; a variation of nutritional state in his group of patients different from that in ours could explain this apparent discrepancy.

TABLE I. ACETYLCHOLINESTERASE ACTIVITY IN NORMAL AND PATHOLOGICAL SERA

| | NO. | MEAN | STANDARD DEVIATION | SIGNIFICANT DIFFERENCE |
|---|---|---|---|---|
| Normal subjects | 40 | 0.86 | 0.21 | |
| Male | 20 | 0.95 | 0.24 | $p = < .01$ |
| Female | 20 | 0.78 | 0.12 | |
| Portal cirrhosis, proved or unequivocal | 46 | 0.31 | 0.15 | |
| Acute viral hepatitis | 11 | 0.58 | 0.18 | |
| Benign obstructive jaundice, uncomplicated | 15 | 0.61 | 0.13 | |
| Abdominal carcinoma, proved | 21 | 0.44 | 0.17 | |
| Cachectic | 9 | 0.32 | 0.16 | $p = < .01$ |
| Noncachectic | 12 | 0.53 | 0.12 | |
| Cachexia (noncirrhotic) | 22 | 0.37 | 0.21 | |
| Malignancy | 9 | 0.32 | 0.16 | $p = > .5$ |
| No malignancy | 13 | 0.40 | 0.24 | |

Of the group of patients with proved intra-abdominal carcinoma, the mean acetylcholinesterase activity in the sera of those with good nutritional status was significantly greater than that from patients whose malignancy was accompanied by cachexia. No significant difference in mean enzyme activity

TABLE II. CORRELATION OF ACETYLCHOLINESTERASE ACTIVITY AND ALBUMIN CONCENTRATION IN PATHOLOGIC SERA AND IN ASCITIC FLUID

(Summary of Statistical Data Derived From Figs. 1 and 2)

| | NO. OF PATIENTS | NO. OF OBSERVATIONS | CORRELATION COEFFICIENT (R) | SIGNIFICANCE (P) |
|---|---|---|---|---|
| All sera, initial values | 118 | 118 | +0.748 | $< 0.01$ |
| All sera, all values | 118 | 294 | +0.731 | $< 0.01$ |
| Normal sera | 20 | 20 | −0.114 | $> 0.50$ |
| Pathologic sera, initial values | 98 | 98 | +0.656 | $< 0.01$ |
| Pathologic sera, all values | 98 | 272 | +0.687 | $< 0.01$ |
| Cirrhotic sera, initial values | 42 | 42 | +0.645 | $< 0.01$ |
| Cirrhotic sera, all values | 42 | 141 | +0.732 | $< 0.01$ |
| Hepatitis sera, initial values | 11 | 11 | +0.566 | $> 0.05$ |
| Hepatitis sera, all values | 11 | 70 | +0.735 | $< 0.01$ |
| Other pathologic sera,* initial values | 45 | 45 | +0.563 | $< 0.01$ |
| Other pathologic sera,* all values | 45 | 61 | +0.502 | $< 0.01$ |
| Ascitic fluid† (Fig. 1) | 20 | 22 | +0.794 | $< 0.01$ |

*Includes 12 patients with benign obstructive jaundice, 16 patients with intra-abdominal carcinoma, and 17 patients with miscellaneous disorders, but none with the nephrotic syndrome.

†Includes ascitic fluid from 13 patients with portal cirrhosis, 1 with biliary cirrhosis, 4 with intra-abdominal carcinomatosis, and 2 patients with ascites secondary to congestive failure. Data from 7 other samples of ascitic fluid not included (see text).

was found between the sera of patients with cachexia secondary to carcinoma and the sera of patients with severe malnutrition due to other causes. The enzyme values in patients with carcinoma who were in apparently good nutritional state were significantly lower than in normal individuals; a corresponding depression of the serum albumin concentration was noted in these patients. The selective lowering of the serum albumin concentration in malignant states has been well documented.[28-30]

These observations support the contention[16-20] that nutritional status is the prime factor determining the level of the serum enzyme activity in pa-

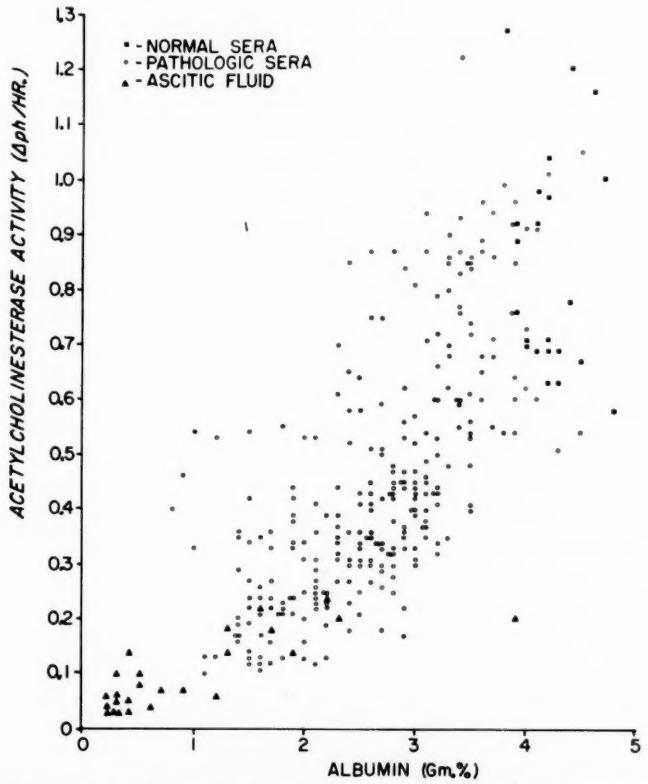


Fig. 1.—Relationship of acetylcholinesterase activity to albumin concentration in normal and pathologic sera, and in ascitic fluid.

tients without parenchymal liver disease or the nephrotic syndrome. But the data also suggest that malignancy even in the absence of malnutrition depresses the serum acetylcholinesterase activity. Levine and Hoyt[15] have previously shown that a broad group of patients with various types of malignant disease have average serum enzyme values less than those for normal subjects.

*Correlation Between Serum Acetylcholinesterase Activity and Albumin Concentration.*—A positive correlation was found between the acetylcholinesterase activity and the albumin concentration of abnormal sera (Fig. 1 and Table II). This positive correlation is statistically significant for each group of abnormal

sera studied, with the exception of the initial values of the acute hepatitis group where the number of patients is so small that the correlation is of questionable significance. The absence of correlation between the enzyme activity and the albumin concentration in normal sera agrees with the findings of previous investigators.[3]

Because of the varying number of follow-up determinations obtained during the clinical course of the patients studied and the inconstant time interval between these determinations, the statistical relationship between the initial and subsequent values cannot be interpreted with assurance other than noting that the correlation coefficients are similar (Table II). Fig. 2, however, which

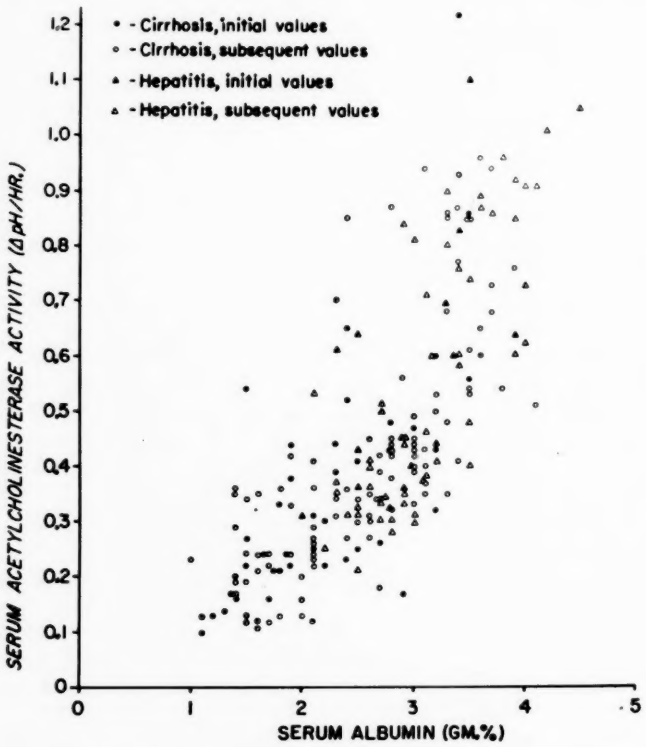


Fig. 2.—Relationship of acetylcholinesterase activity to albumin concentration in the sera of patients with portal cirrhosis or viral hepatitis.

depicts only determinations in sera from patients with parenchymal liver disease and which distinguishes initial and subsequent values, suggests that there is no essential difference in the relationship of enzyme activity to albumin concentration when follow-up determinations are compared with initial ones. This observation indicates that the serial determinations of serum acetylcholinesterase activity in the individual patients studied paralleled in time the variations in serum albumin concentration, rather than being preceded by them[1, 2] or depicting episodes of "biochemical relapse" not also reflected by the serum albumin.[6, 7] Fig. 3 illustrates this temporal parallelism between the serum

albumin concentration and the acetylcholinesterase activity in serial determinations obtained during the hospital course of seven of eight patients with parenchymal liver disease, and during the experimental administration of cortisone to one normal male. Case 9 in Fig. 3 is one of the two exceptions to the pattern of temporal parallelism that has been found in following twenty-five patients with multiple serial determinations.

*The Acetylcholinesterase Activity of Ascitic Fluid.*—Acetylcholinesterase activity and albumin concentration were determined in 29 specimens of ascitic

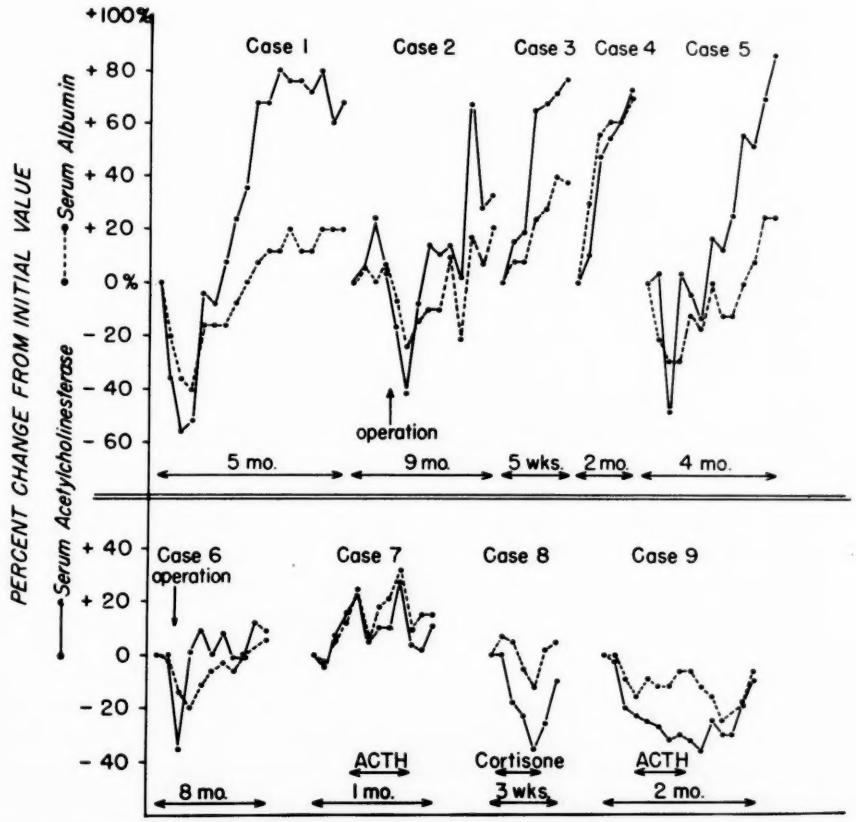


Fig. 3.—Relationship of acetylcholinesterase activity to albumin concentration in serial serum specimens from nine patients (for details, see text).

fluid collected from 26 patients, the majority with advanced portal cirrhosis. In one sample there was no detectable enzyme activity. Six other samples were omitted from consideration either because the enzyme activity was 0.03 units or less, or because the albumin concentration was below 0.2 Gm. per cent, or both; such values were considered too low to permit accurate measurement with the methods employed. The determinations from the remaining 22 specimens are presented in Fig. 1, and the derived statistical data are shown in Table

II. Despite the small number of samples, there is a positive correlation which is statistically significant between the albumin concentration and the enzyme activity in the ascitic fluid samples.

The serum-ascitic fluid ratio for albumin concentration is plotted against the same ratio for enzyme activity in Fig. 4. The points, with two moderate exceptions, lie close to the diagonal line representing identity of the two ratios. This relationship is statistically significant.

## DISCUSSION

The data presented in Table I indicate that the measurement of the serum acetylcholinesterase activity may be of diagnostic value only to the extent of separating cases of portal cirrhosis from the remainder of the group of hepatobiliary diseases, and then only in the absence of cachexia. Two considerations suggest that the test may not be helpful even to this limited degree:

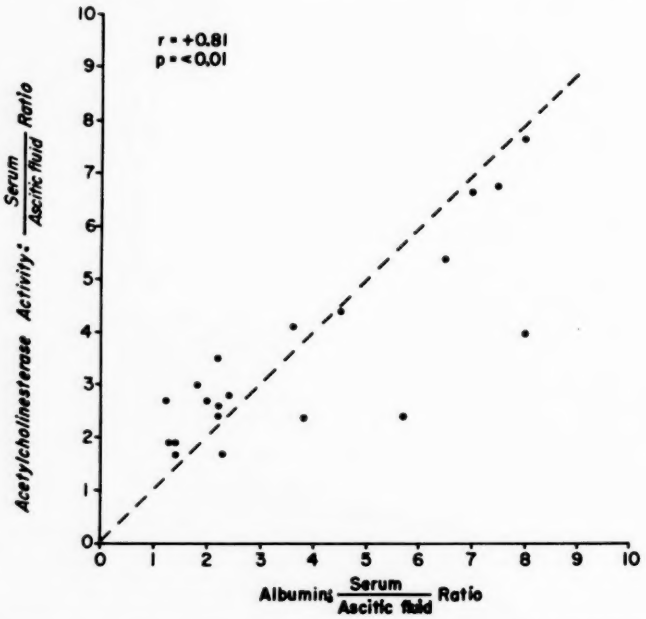


Fig. 4.—Relationship of the serum-ascitic fluid ratio for acetylcholinesterase activity to the serum-ascitic fluid ratio for albumin concentration.

(1) A marked overlap of the individual values for enzyme activity between all groups of pathologic sera was found, as has been reported by others.[6, 13] In the present series it was observed, in addition, that those patients presenting diagnostic problems most frequently had serum enzyme levels lying in the uncertain overlap area.

(2) In a compilation of data such as presented in Table I, values obtained from patients who present little diagnostic difficulty usually predominate, as is true in the present series. Cases in which the diagnosis was not initially apparent were excluded, unless subsequently the true nature of the disease

was determined pathologically or became clinically unequivocal. It was also necessary to discard those cases of hepatobiliary disease which did not clearly fall into any single grouping, or in which more than one disease process was suspected. The deductions which may be drawn from the data presented in Table I, therefore, may not apply to patients in whom the diagnosis is complicated or obscure, and cannot be said to be helpful in the differential diagnosis of the conditions studied.

The conclusion that the measurement of serum acetylcholinesterase activity does not provide information of diagnostic value in hepatobiliary disease not available from other laboratory procedures confirms the findings of Mann and co-workers;[13] it is further substantiated by the positive correlation noted between the enzyme activity and the albumin concentration in abnormal sera (Fig. 1). The consistency of this positive correlation throughout all observations, both initial and serial, supports the essential parallelism found between the two determinations during the course of disease in single patients (Fig. 3). This temporal parallelism, however, does not obviate the value of serial determinations of the serum acetylcholinesterase activity in assessing the progress of certain patients. It will be noted in Fig. 3 that the ordinate values for both albumin concentration and for enzyme activity are plotted in terms of *per cent change from initial value*. In four of the eight cases demonstrating parallel changes in Fig. 3, the serum acetylcholinesterase activity shows a much greater change from the initial value than does the albumin concentration (Cases 1, 3, 5, and 8); in none does the changing albumin concentration overshadow the parallel variation in enzyme activity. In these four patients, the acetylcholinesterase activity provided a definite indication of changing liver function at a time when the albumin concentration, although varying in the same direction, had not yet shown an unequivocal change. This phenomenon was noted in half of the twenty-five cases followed with serial determinations; in none did changes in the enzyme activity precede the changes in albumin concentration by a significant interval, or vice versa. In the two cases in which temporal parallelism of the two determinations did not exist, no relationship of any sort was apparent (Fig. 3, Case 9).

Serial determinations of serum acetylcholinesterase activity were found to be particularly useful in assessing the progress of patients with portal cirrhosis, a disease in which it is well accepted that the serum albumin concentration is one of the most valuable of the standard liver function tests for following the course of the disease.[21-23] Since a change of prognostic importance may be apparent in the enzyme activity level at a time when the simultaneous change in albumin concentration is of doubtful significance, while the converse situation has not been observed, serial determinations of the serum acetylcholinesterase activity appear to be of even greater value in portal cirrhosis than the albumin concentration. This was also found in certain cases of acute viral hepatitis, where both tests appear to be of value in following the acute phase in the more severely ill patients.[7, 24]

In ascitic fluid, as in serum, there is a positive correlation between the enzyme activity and the albumin concentration (Table II). The observation that

the serum-ascitic fluid ratio for acetylcholinesterase activity parallels the same ratio for albumin concentrations (Fig. 4) provides confirmatory evidence that, frequently, the serum protein pattern is reflected with only minor alterations in ascitic fluid.[25-27, 31]

The absence of correlation between the serum albumin concentration and the acetylcholinesterase activity in nephrosis has been noted by various investigators.[2, 3] In that syndrome, a high serum enzyme activity has been demonstrated despite moderate to marked hypoalbuminemia. No such patients have been studied in this report.

## SUMMARY OF CASES SHOWN IN FIG. 3

CASE 1.—J. D., a 43-year-old woman with advanced Laennec's cirrhosis, was admitted in hepatic coma. She was ambulatory during last three months of observation. The patient expired from bleeding esophageal varices; these diagnoses were confirmed by autopsy. Initial serum values: acetylcholinesterase 0.25 units, albumin 2.5 Gm. per cent.

CASE 2.—B. B., a 50-year-old woman, had hyperadrenalcorticism and viral hepatitis. These diagnoses were confirmed by liver biopsy at operation (left adrenalectomy). She had a severe biochemical relapse of hepatitis postoperatively. Initial serum values: acetylcholinesterase 0.36 units, albumin 2.9 Gm. per cent.

CASE 3.—F. L., a man aged 50 years, had acute viral hepatitis with rapid recovery (clinical diagnosis). Initial serum values: acetylcholinesterase 0.43 units, albumin 2.5 Gm. per cent.

CASE 4.—C. R., a man aged 49 years, was suffering from acute fatty cirrhosis. Diagnosis was confirmed by needle liver biopsy. Initial serum values: acetylcholinesterase 0.44 units, albumin 2.3 Gm. per cent.

CASE 5.—F. F., a 38-year-old man with far-advanced Laennec's cirrhosis, was admitted to the hospital in hepatic stupor (clinical diagnosis). He was semiambulatory at the end of the period of observation. Initial serum values: acetylcholinesterase 0.23 units, albumin 2.4 Gm. per cent.

CASE 6.—E. N., a man aged 67 years, had postnecrotic cirrhosis with esophageal varices; this diagnosis was confirmed by biopsy taken during operation (direct portacaval shunt). Initial serum values: acetylcholinesterase 0.86 units, albumin 3.5 Gm. per cent.

CASE 7.—J. L., a woman aged 32 years, was suffering from acute viral hepatitis of moderate severity (clinical diagnosis). She was treated with ACTH. Initial serum values: acetylcholinesterase 0.83 units, albumin 3.4 Gm. per cent.

CASE 8.—C. F., a man aged 36 years, was a normal "control" receiving cortisone. Measured hemodilution was insufficient to account for changes shown. Initial serum values: acetylcholinesterase 0.92 units, albumin 4.1 Gm. per cent.

CASE 9.—M. N., a woman aged 20 years, had chronic viral hepatitis (clinical diagnosis). She received ACTH during the period indicated, with no clinical change. Initial serum values: acetylcholinesterase 0.44 units, albumin 3.2 Gm. per cent.

## SUMMARY

A difference of statistical significance in mean serum acetylcholinesterase activity, as measured by the Michel method, was demonstrated between normal sera and sera from patients with various diseases. A statistically significant difference was found between the serum enzyme activity in advanced portal cirrhosis and that in acute hepatitis, benign obstructive jaundice, and intra-

abdominal carcinoma without cachexia; there was no significant difference between patients in the latter three groups. No difference of statistical significance in serum acetylcholinesterase activity was observed between patients with intra-abdominal malignancy with cachexia and patients with severe malnutrition but without carcinoma.

A positive correlation of statistical significance was found in pathologic sera* between acetylcholinesterase activity and albumin concentration as determined by the Reinhold salting-out method. No such correlation existed for normal sera.

Serial determinations of the serum acetylcholinesterase activity in single patients with a variety of diseases showed fluctuations which paralleled in time those of the serum albumin concentration. The serum enzyme determinations often showed a greater per cent change from the initial value at any given time during the clinical course than did the albumin concentration. Serial measurements of serum acetylcholinesterase activity frequently revealed a change of prognostic significance at a time when the serum albumin concentration, while tending to vary in the same direction, showed merely an equivocal fluctuation.

A positive correlation of statistical significance was found between the acetylcholinesterase activity and the albumin concentration of ascitic fluid of varied etiology. A positive correlation between the serum-ascitic fluid ratio for acetylcholinesterase activity and that for albumin concentration was also observed.

## CONCLUSIONS

Measurement of the serum acetylcholinesterase activity does not provide information of value in the differential diagnosis of hepatobiliary disease not available from established laboratory procedures.

Serial determination of serum acetylcholinesterase activity appears to be useful in assessing the course of patients with portal cirrhosis or with severe viral hepatitis in the acute phase. Although paralleling in time the serum albumin, changes of prognostic importance are frequently evident in the changing serum enzyme activity level at a time when the simultaneous change in serum albumin concentration appears of doubtful significance.

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# OBSERVATIONS ON PLASMA GLUTAMIC ACID

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IN AN earlier paper,[1] it was reported that subjects with malignant growths exhibited a significant elevation in their plasma level of glutamic acid. No such elevation was observed in subjects with benign growths. It was suggested that, although an abnormal level of plasma glutamic acid was not, per se, a specific sign of cancer, the determination of this metabolite's concentration in plasma might be useful as a diagnostic means of distinguishing benign from malignant growths prior to operation. It was stated that abnormal levels had been observed in cases of hyperemesis gravidarum. Subsequent work in this laboratory has shown that an elevated plasma glutamic acid level may be observed in clinical conditions other than malignancy and hyperemesis gravidarum.

## METHODS

For the determination of glutamic acid in plasma, the chemical method of Prescott and Waelsch[2] as modified in a later report[3] was employed, since this method has been found to offer a high degree of specificity. In all cases venous blood samples were drawn from the subjects, heparinized, and the plasma separated. Since the effect of the ingestion of a meal on the plasma concentration of glutamic acid had been found to be negligible, blood samples were taken from subjects in the nonfasting state in most cases. In order to evaluate the plasma glutamic acid levels found for the subjects investigated, comparisons were made with the levels reported for normal human beings.[4] It should be noted again that 26 normal male subjects had a mean plasma glutamic acid level of 1.2 mg. per cent, with a range of 0.7 to 2.0 mg. per cent, and that of 51 normal female subjects was 1.0 mg. per cent, with a range of 0.5 to 1.8 mg. per cent.[4] The data reported here for subjects for clinical conditions represent the results of determinations on both sexes. The investigations have included an extension of the malignant series with an evaluation according to type and location of malignant tumor, and also determinations on a number of other clinical conditions. A further study was carried out to investigate the intraindividual variation in disease. In a separate study, determinations of plasma glutamic acid were made on infants at birth (cord blood) and maternal blood during labor.

## RESULTS

Data for a heterogeneous group of patients are given in Table I. Values for different locations and types of malignancy are shown in Table II. The standard deviation of plasma glutamic acid levels in persons with malignant growths was found to be 1.7 mg. per cent. Application of a t test shows that the elevation in the mean plasma level of glutamic acid in malignancy is highly significant. Further, it was observed that 87 per cent of the subjects studied exhibited plasma glutamic acid levels within the range provided by the mean ± the standard deviation.

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TABLE I. PLASMA GLUTAMIC ACID LEVELS IN VARIOUS CLINICAL CONDITIONS

| CONDITION | NUMBER OF SUBJECTS | RANGE (MG. PER CENT) | MEAN (MG. PER CENT) |
|---|---|---|---|
| Malignancy | 156 | 1.0-11.9 | 3.2 |
| Benign tumors | 21 | 0.5- 1.7 | 1.2 |
| Inflammatory infections | 15 | 1.1- 5.7 | 3.2 |
| Coronary thrombosis | 11 | 0.9- 5.0 | 3.0 |
| Hypertension | 12 | 1.5- 7.8 | 3.6 |
| Diabetes | 3 | 2.3- 3.1 | 2.5 |
| Epilepsy | 2 | 2.7- 3.4 | 3.0 |
| Myasthenia gravis | 1 | 1.6 | 1.6 |
| Peptic ulcer | 3 | 1.1- 3.8 | 2.8 |
| Anemia | 3 | 1.6- 2.8 | 2.5 |
| Rheumatoid arthritis | 1 | 1.7 | 1.7 |
| Tuberculosis | 2 | 1.4- 2.7 | 2.1 |
| Liver cirrhosis | 7 | 1.3- 5.5 | 3.8 |
| Emphysema | 1 | 3.3 | 3.3 |
| Myelomatosis | 1 | 1.2 | 1.2 |
| Hyperemesis gravidarum | 15 | 0.8- 5.9 | 2.8 |
| Toxemia of pregnancy | 5 | 1.0- 1.8 | 1.5 |
| Septicemia | 1 | 2.2 | 2.2 |

TABLE II. PLASMA GLUTAMIC ACID LEVELS IN VARIOUS CLASSIFICATIONS OF MALIGNANCY

| LOCATION OR TYPE OF MALIGNANCY | NUMBER OF SUBJECTS | RANGE (MG. PER CENT) | MEAN (MG. PER CENT) |
|---|---|---|---|
| Vulva | 2 | 1.7- 3.0 | 2.4 |
| Cervix | 18 | 1.8- 6.8 | 2.7 |
| Ovaries | 5 | 2.5- 3.1 | 2.8 |
| Testicles | 1 | 2.4 | 2.4 |
| Uterus | 6 | 2.1- 4.6 | 3.1 |
| Prostate | 2 | 2.7- 4.0 | 3.3 |
| Breast | 21 | 1.2-10.0 | 2.9 |
| Stomach | 10 | 2.2- 5.0 | 3.3 |
| Caecum | 1 | 2.5 | 2.5 |
| Bowel | 2 | 2.7 | 2.7 |
| Colon | 2 | 3.8- 4.2 | 4.0 |
| Gastrointestinal tract | 3 | 2.5- 3.2 | 2.9 |
| Rectum | 4 | 1.9- 4.0 | 3.1 |
| Chest | 3 | 3.0- 4.8 | 3.6 |
| Lungs | 4 | 1.6- 3.2 | 2.3 |
| Bronchi | 8 | 2.3- 8.2 | 3.7 |
| Thyroid | 1 | 5.6 | 5.6 |
| Pancreas | 5 | 1.9- 9.4 | 6.1 |
| Liver | 1 | 7.7 | 7.7 |
| Kidney | 1 | 4.8 | 4.8 |
| Lip | 7 | 1.3-11.8 | 3.4 |
| Palate | 1 | 3.3 | 3.3 |
| Tongue | 3 | 1.8- 1.9 | 1.9 |
| Tonsil | 1 | 1.4 | 1.4 |
| Floor of mouth | 1 | 1.2 | 1.2 |
| Gingiva | 1 | 2.4 | 2.4 |
| Neck | 2 | 2.0- 2.2 | 2.1 |
| Cheek | 2 | 1.3- 2.4 | 1.8 |
| Nose | 3 | 1.4- 2.9 | 2.4 |
| Ear | 1 | 4.3 | 4.3 |
| Lymphoblastoma | 5 | 1.6- 4.0 | 2.4 |
| Leukemia | 6 | 1.0- 8.8 | 3.2 |
| Hodgkin's disease | 7 | 1.7- 4.0 | 2.3 |
| Unclassified | 22 | 1.4- 6.8 | 2.8 |

Determinations of plasma glutamic acid have been made on cord blood taken from 22 infants at birth. The mean level found was 9.5 mg. per cent with a range of 3.7 to 14.9 mg. per cent, a marked elevation over the values obtained for normal subjects. Analyses were carried out on the plasma of 11 of the mothers during labor, and the mean level was found to be 4.3 mg. per cent, with a range of 1.7 to 7.3 mg. per cent, again a marked elevation over the levels obtained for normal subjects. It is interesting that values for maternal blood were generally much less than those for cord blood.

## DISCUSSION

Several conclusions are evident from the data presented. An extension of the malignant series has confirmed the earlier observation that plasma glutamic acid concentrations are elevated in most persons with malignancy. However, such an elevation was found in several other clinical conditions studied, and may well be found in others not studied. This finding does not invalidate the suggestion that, if a subject is known to have a growth, a plasma glutamic acid determination may be useful in distinguishing the type of tumor prior to surgery. However, these results do indicate the need for a careful examination of the subject's clinical history prior to interpretation of the results of the plasma glutamic acid determination. No correlation could be made between the plasma glutamic acid level and the type or location of the malignancy, and it would seem that the level of plasma glutamic acid can offer no aid in estimating the extent of the malignancy. As would be expected with any biological substance, considerable intraindividual variation was noted in the plasma glutamic acid level of subjects with clinical conditions, but in only one case did a subject with an initial abnormally high plasma glutamic acid concentration exhibit a normal level when a second sample was taken within two weeks.

The elevated levels noted in maternal blood during labor are of interest, since it has been reported from this laboratory that plasma glutamic acid levels are not elevated in any of the three trimesters of pregnancy.[4] It appears that the elevation in this metabolite during labor is a true consequence of metabolic changes during this phase of pregnancy. It has been known for some years that the concentration of total amino acids in fetal whole blood and plasma is elevated above that found for normal adults, and, further, that the level of amino acids in fetal blood is higher than in maternal blood.[5] It is not surprising, therefore, that the plasma glutamic acid concentration in cord blood was found to be elevated above that of the normal series. Since in every case in which maternal and cord blood samples were taken from a mother and her infant the plasma glutamic acid concentration of the cord blood exceeded that of the maternal blood, it would seem that the cord blood sample was representative of the infant's own blood, not that of the mother.

The significance of an increased amount of glutamic acid in blood plasma is not apparent at present. Glutamic acid is believed widely to be a very active metabolite. It may well be that the presence of abnormal amounts in blood is indicative of a metabolic disturbance. In the case of cancer patients, particularly, it would be useful to ascertain the responsible mechanism.

SUMMARY

Determinations of plasma glutamic acid concentrations in several clinical conditions have shown that not only are elevations found in malignancy and in hyperemesis gravidarum, as previously reported, but have also been observed in several other clinical conditions. An elevation in plasma glutamic concentration has been found in infants at birth (cord blood) and in maternal blood during labor, the former elevation exceeding the latter. Subjects with clinical conditions were found to exhibit considerable intraindividual variation.

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# THE POTASSIUM, SODIUM, AND CALCIUM CONTENT OF GASTRIC JUICE

## I. Normal Values

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GASTROINTESTINAL physiologists and clinical investigators have devoted their attention mainly to the acidity and peptic activity of gastric juice as indicators of the function of the gastric mucosa and its reaction to various stimuli.

Little consideration has been paid to the significance of other cations present in the gastric juice. Until recently estimations of potassium, sodium, and calcium in biological fluids have depended upon tedious and laborious chemical methods. As a result, few reports on these electrolytes in human gastric juice are available.[1-3] The introduction of flame photometry has made possible a technique that is simple, rapid, and capable of accuracy superior to chemical determinations.

### MATERIALS AND METHODS

Samples of gastric juice were obtained from 50 healthy male students and staff, free from concurrent infection, in the resting state after an overnight fast. Specimens were completely removed through a nasogastric tube at ten or fifteen minute intervals up to three hours; short interval sampling was considered necessary in order to limit back diffusion of ions from the stomach lumen and to exclude bile-stained samples from analysis. Salivary contamination was minimized by collecting saliva separately by continuous aspiration or expectoration.

The specimens were immediately filtered (some specimens were centrifuged in order to determine the percentage volume of mucin and for cation estimations on the separated mucin) and placed in hard glass containers for cation determination. Free and total acidity was titrated using Topfer's reagent and phenolphthalein with buffered standards at pH 3.5 and 8.5, respectively, and the Beckman pH meter to check the end point of the titration. Total chloride was estimated by the Volhard method.

The following chemical methods were used to compare with the flame analyses: (a) sodium by gravimetric estimation of the sodium zinc uranyl acetate precipitate; (b) potassium in ashed samples by a modification of the cobaltinitrite method described for urine[4] (an aliquot of the dissolved ash was used for flame analysis to compare with the results by dilution); and (c) calcium after neutralization of the gastric juice followed by oxalate precipitation for sixteen to twenty-four hours (shorter periods were found not to give complete precipitation on recovery experiments) and permanganate titration or ceric sulfate with thiosulfate back titration.

*Flame Spectrophotometric Analysis.*—Flame analysis was performed with a Perkin-Elmer 52A flame spectrophotometer,[5] using the internal standard technique to measure light intensity ratios and a modification of methods described for serum.[6] Since this instrument can also be used to measure light emission directly, it has been possible to compare results by this method.[7-9]

The emission intensity of an ion in the flame is dependent on the amount of ion delivered to the flame in unit time, flame temperature, and the effects of interionic action and organic

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substances. Constant atomizing conditions were obtained by atomizing a fixed volume (3 or 5 ml.) of diluted gastric juice at 18 to 20° C. with compressed air at 10 pounds per square inch, and taking photometer readings at a fixed level in the atomizer. Acetylene was supplied at a constant pressure of 4 pounds per square inch (6 pounds per square inch for calcium estimations) to obtain a uniform flame temperature.

Optimum operating conditions were found by atomizing gastric juice suitably diluted to fall within the maximum sensitivity range of the instrument for the particular ion. After preliminary tests, dilutions of 1:20 or 1:50 for sodium and 1:20 for potassium gave the most accurate results. Undiluted juice and also samples, prepared by oxalate precipitation of calcium and redissolved in 0.05N HCl, were used for calcium determinations. Lithium as internal standard was added to make the final concentration the same as for the standard solutions.

Since 7 per cent alcohol orally was used as a gastric stimulant in some experiments, the effect of adding 1 to 5 per cent alcohol to suitable cation solutions or gastric juice specimens was studied. By lowering surface tension and increasing the vapor yield on atomization, alcohol enhanced the direct light intensity measurements of sodium and potassium up to 3 per cent, and calcium between 5 to 20 per cent, but produced no effect when light intensity ratios were estimated with the internal standard. It was not found possible to correct for the varying alcohol concentration of specimens with the direct method.

*Sources of Flame Interference.*—Within the range of potassium, sodium, and calcium values in gastric juice, the light emission of an ion by direct measurement may be depressed up to 5 per cent by the interionic action of other ions. These effects were compensated by the use of the internal standard, except that sodium enhanced the light emission of potassium about 1 per cent when the Na/K ratio exceeded 5.[10] This occurred in about 20 per cent of all specimens, whose ratios ranged from 1 to 13.

The presence of acid depressed the light emission of cations. Since the maximum gastric acidity does not exceed 150 meq. per liter, the acid present caused no interference in gastric juice diluted for sodium and potassium estimations (Table I); however, in the determination of calcium on undiluted juice, the internal standard provided effective compensation for the considerable error found on measuring the direct intensity.

TABLE I. THE EFFECT OF HYDROCHLORIC ACID ON THE LIGHT EMISSION OF SODIUM, POTASSIUM, AND CALCIUM

| CONCENTRATION OF ACID (MEQ./L.) | DIRECT EMISSION | | | INTERNAL STANDARD EMISSION | | |
|---|---|---|---|---|---|---|
| | Na* | K | Ca | Na | K | Ca |
| | (DECREASE PER CENT) | | | (DECREASE PER CENT) | | |
| 25 | 1 | 1 | 3 | 0.5 | 0 | 1 |
| 50 | 1 | 2 | 4 | 1 | 1 | 2 |
| 75 | 4 | 3 | 7 | 2 | 2 | 4 |
| 100 | 6 | 5 | 8 | Nil | | |
| 150 | 7 | 6 | 10 | | | |
| 200 | 8 | 8 | 12 | | | |
| 500 | 12 | 11 | 15 | | | |

*Sodium solutions, 1.0 and 2.0 meq. per liter; potassium, 0.5 and 1.0 meq. per liter; calcium, 2.0 and 5.0 meq. per liter.

Lithium (671 mμ) added as an internal standard to all solutions is subject to the same influences in the flame as sodium and potassium, with some slight variation in respect to calcium. The error due to the use of lithium in obtaining light intensity ratios was found to be negligible (< 1 per cent under all conditions) compared with those resulting from the compounding of atomization and flame variables in the direct technique.

*Isolation and Calibration of Emissions.*—The flame emission was isolated by a 0.5 mm. spectral slit at 589 mμ for sodium, 766 mμ for potassium, and 554 mμ for calcium. The sodium and potassium slits do not pass light emitted by other ions in contrast to filter photometers[11, 12]; however, the wave length used for calcium is a band spectrum, and sodium was found to increase light intensities up to 4 per cent at this wave length in concentrations from 0 to 100 meq. per liter. This may be adequately corrected by using standard calcium solutions with added sodium (vide infra).

Standard solutions for calibration curves and standardization were prepared from spectroscopically pure chlorides (Johnson, Matthey and Co., London) and kept in No-Sol-Vit (Perkin-Elmer Corporation) bottles. Calibration curves for sodium were made in the range 0 to 1 meq. per liter and 0 to 2 meq. per liter, with standard solutions at 0.1 meq. per liter interval and containing 5 meq. per liter lithium for the internal standard procedure; for potassium 0 to 0.5 meq. per liter, and 0 to 1 meq. per liter, with standard solutions at 0.05 meq. per liter interval and containing 5 meq. per liter of lithium.

The calcium calibration covered the range of 0 to 5 meq. per liter, with 2.5 meq. per liter of lithium added for internal standard estimations. The sodium emission passing the slit was compensated by preparing the calibration series with sodium added in the concentration of 0 to 100 meq. per liter at 20 meq. per liter intervals and using the appropriate standard once the sodium content of the specimen had been determined. The correction in the direct technique for the acid content of the gastric juice was made from the interference curve (Table I).

Calibration curves in these ranges were essentially linear for the internal standard procedure, and standard solutions prepared at intervals over the past three years gave readings of standard points that did not differ by more than one dial division. With the direct procedure, the meter readings of standards showed some scatter and the meter needle an oscillation up to 1½ divisions during atomization of the sample.

The electrical circuit and flame were started fifteen to twenty minutes before operation, and 200 ml. of glass distilled water were atomized to stabilize the temperature of the atomizer chamber. Each specimen was atomized in duplicate and read off from the calibration curve. A check result was obtained by bracketing the unknown with two standard solutions. These readings were within 2 per cent of each other for all sodium and potassium estimations, and within 3 per cent of each other for most calcium values.

*Error of Flame and Chemical Analysis.*—The accuracy of the methods employed has been evaluated. Recovery from prepared standards was greatest and with the lowest coefficient of variation by internal standard flame analysis, and the standard deviation of duplicate estimations was also lowest by this technique. In Table II a statistical analysis of replicate estimations on the same gastric juice sample by the different methods is recorded.

The value of flame spectrophotometric methods lies in the great saving of time compared with chemical methods. Further, internal standard flame analysis was found to be more accurate and reliable than established chemical methods for sodium and potassium, and of a comparable accuracy to the classical chemical estimation of calcium. The cation values recorded here were obtained by internal standard flame analysis.

## Results

### Electrolyte Partition in Gastric Juice

Fifty fasting samples showed the following electrolyte pattern: free acid values ranged from 0 to 66 (mean, 32) meq. per liter, sodium from 18.7 to 69.5 (mean, 49) meq. per liter, and potassium from 6.4 to 16.6 (mean, 11.6) meq. per liter. Calcium levels varied from 2.0 to 4.8 meq. per liter, giving a mean of 3.6 meq. per liter.

The overnight sample of fasting juice, apart from regurgitation of duodenal contents, may be contaminated with swallowed saliva or postnasal secretions. The effect of this was studied in 15 subjects, who were followed for one to two hours by periodic removal of gastric juice and continuous salivary aspiration. During these periods the gastric juice sodium, free acid, and total chloride showed irregular fluctuations, and the potassium and calcium contents a variation that did not exceed 2.2 and 0.5 meq. per liter, respectively. The potassium values for 82 interval specimens ranged from 6.3 to 13.4 (mean, 9.7) meq. per liter, sodium 16.0 to 58.6 (mean, 42) meq. per

liter, and 44 specimens gave calcium values of 2.2 to 4.6 (mean, 3.3) meq. per liter. The gastric juice potassium and calcium average for each subject were less than the subject's fasting value ($P < 0.05$).

The salivary potassium and calcium values obtained, 8.8 to 28.6 (mean, 20.4) and 4.0 to 8.3 (mean, 6.5) meq. per liter, respectively, were in all instances greater than the corresponding gastric juice values. Sodium levels in the saliva of these subjects ranged from 16 to 78 (mean, 44) meq. per liter. These results indicated that, since fasting gastric juice was contaminated with a variable amount of salivary secretion, it was necessary to observe one or more preinjection periods after the removal of the fasting secretion to provide the base line to interpret cation alterations.

TABLE II. STATISTICAL ANALYSIS OF ERROR OF TECHNIQUE: 20 ESTIMATIONS BY FLAME ANALYSIS AND 10 BY CHEMICAL METHODS ON THE SAME SPECIMEN OF GASTRIC JUICE

| ION | SUBJECT AND SPECIMEN | METHOD | MEAN (MEQ./L.) | STANDARD DEVIATION (MEQ./L.) | COEFFICIENT OF VARIATION (PER CENT) |
|---|---|---|---|---|---|
| Na | C. C. C. 3I | Flame analysis, internal standard | 46.3 | 0.45 | 0.97 |
| | | Flame analysis, direct | 46.2 | 0.86 | 1.86 |
| | | Chemical | 45.8 | 1.73 | 3.78 |
| | D. S. 6I | Flame analysis, internal standard | 11.3 | 0.15 | 1.36 |
| | | Chemical | 10.8 | 0.48 | 4.48 |
| K | J. F. v Z. 31 | Flame analysis, internal standard | 11.6 | 0.17 | 1.43 |
| | | Flame analysis, direct | 11.5 | 0.24 | 2.07 |
| | | Chemical | 12.0 | 0.39 | 3.22 |
| | C. H. L. 4I | Flame analysis, internal standard | 4.6 | 0.072 | 1.57 |
| | | Flame analysis, internal standard, ashed at 450° C. | 4.6 | 0.10 | 2.22 |
| | | Chemical | 4.8 | 0.163 | 3.40 |
| Ca | T. P. 4I | Flame analysis, internal standard | 1.88 | 0.043 | 2.29 |
| | | Flame analysis, internal standard, oxalate precipitation | 1.72 | 0.063 | 3.68 |
| | | Chemical | 1.67 | 0.091 | 5.49 |

### EFFECT OF GASTRIC SECRETORY STIMULANTS

Subjects were given histamine, 0.1 mg. per 10 kg. of body weight subcutaneously (14 subjects); 50 ml. of 7 per cent alcohol followed one to one and one-half hours later by 0.1 mg. histamine per 10 kg. of body weight subcutaneously (15 cases); and 1 unit of insulin per 10 kg. of body weight intravenously (10 students, who had been on a high carbohydrate diet for several days prior to the experiment).

(a) *Histamine Cases.*—In these cases the volume, acidity, and total chloride increased rapidly to reach a maximum thirty to sixty minutes after injection. At the height of secretion the volume was 2 to 6 ml. per minute, compared with resting values up to 1.5 ml. per minute. The acidity increased to attain maximum values of 86 to 132 meq. per liter (mean, 116 meq. per liter); there was a concomitant increase in total chloride, the maximum values ranging from 120 to 158 meq. per liter (mean, 148 meq. per liter).

The gastric juice sodium showed in all instances a variable decrease ranging up to 32 meq. per liter from preinjection levels. In 10 cases the content in one or more specimens was less than 25 meq. per liter, and the lowest values recorded were 12 meq. per liter in 3 cases.

In most subjects, the potassium levels showed an initial rise, followed by a fall reaching a maximum in thirty to ninety minutes, with recovery to previous normal levels by one hundred twenty to one hundred eighty minutes.

In all subjects, the calcium levels had dropped sharply fifteen minutes after injection to reach very low values which persisted for the succeeding hour. In eight cases, in one or more specimens at the height of secretion, no calcium could be detected by the methods used. The calcium content recovered to near normal in one hundred twenty to one hundred eighty minutes.

A slight fall in plasma chloride occurred during maximal secretory activity; the changes in plasma potassium and sodium were not statistically significant.

A representative result is recorded in Table III.

TABLE III. THE EFFECT OF HISTAMINE ON GASTRIC SECRETION OF ELECTROLYTES

| TIME | VOLUME | | $Na^+$ | $K^+$ | $ca^+$ | $H^+$ | $cl^-$ |
|---|---|---|---|---|---|---|---|
| (MIN.) | (ML.) | (ML./MIN.) | (MEQ./L.) | | | | |
| Fasting | 38 | | 65 | 13.0 | 3.95 | 16 | 108 |
| −30 | 14 | 1.0 | 59 | 12.4 | | 28 | 110 |
| −15 | 6 | 0.4 | 60 | 12.7 | 3.78 | 35 | |
| *Histamine 0.6 mg. subcutaneously* | | | | | | | |
| 15 | 50 | 3.3 | 40 | 13.6 | 1.50 | 73 | |
| 30 | 58 | 3.9 | 18 | 13.2 | 0.80 | 103 | 145 |
| 45 | 42 | 2.8 | 20 | 11.9 | 1.08 | 129 | |
| 60 | 47 | 3.1 | 12 | 12.3 | 0.60 | 124 | 157 |
| 75 | 20 | 1.3 | 48 | 12.0 | 2.10 | 45 | 115 |
| 90 | 22 | 1.5 | 50 | | | 52 | |
| 120 | 35 | 1.2 | 50 | 13.0 | 3.40 | 25 | 100 |
| 150 | 12 | 0.4 | 55 | | 3.65 | 38 | 110 |

(b) *Alcohol-Histamine Cases.*—Fractional removal of gastric juice after the alcohol stimulus indicated an increased secretion of acid to attain maximum acidity values of 0 to 89 meq. per liter, with concomitant decreases in sodium, potassium, and calcium values. The subsequent response to histamine injection followed the electrolyte pattern described above (Tables III and IV).

(c) *Insulin Cases.*—In general, the volume secreted and acidity increased to reach the greatest values sixty to ninety minutes after injection, and returned to resting values within the next hour. In every case there was a fall in gastric juice potassium, which reached a maximum some sixty to one hundred twenty minutes after injection; this occurred shortly after the lowest blood glucose and plasma potassium levels were reached and the maximum increase in red cell potassium (Table V). The greatest decrease in each individual case ranged from 2.8 to 7.0 meq. per liter of potassium. At the end of the two- to three-hour test period the concentration was still below resting values in all cases.

At the height of secretion sodium values showed a considerable decrease similar to the histamine results; the lowest values found were 4 and 7 meq. per liter (Table IV).

TABLE IV. ALTERATIONS IN ELECTROLYTE PATTERN OF THE GASTRIC JUICE IN HEALTHY MALE ADULTS FOLLOWING VARIOUS STIMULI COMPARED WITH PRESTIMULATION LEVELS

| STIMULUS | NO. OF CASES | SODIUM MAX. CHANGE IN EACH CASE (MEQ./L.) | POTASSIUM MAX. CHANGE IN EACH CASE (MEQ./L.) | CALCIUM MAX. CHANGE IN EACH CASE (MEQ./L.) | FREE ACID MAX. LEVEL IN EACH SUBJECT (MEQ./L.) | FREE ACID MAX. CHANGE IN EACH SUBJECT (MEQ./L.) | TOTAL CHLORIDE MAX. LEVEL IN EACH SUBJECT (MEQ./L.) | TOTAL CHLORIDE MAX. CHANGE IN EACH SUBJECT (MEQ./L.) |
|---|---|---|---|---|---|---|---|---|
| Nil (resting) | 15 | 12 (range) | 2.2 (range) | 0.5 (range) | 20 to 68 (46) | 18 (range) | 60 to 112 (92) | 12 (range) |
| Insulin | 10 | −13 to −35 (−23) | −2.8 to −7.0 (−5.0) | −0.3 to −1.7 (−0.9) | 63 to 119 (92) | +50 to +97 (+62) | 109 to 150 (134) | +15 to +92 (+41) |
| Histamine | 14 | −10 to −32 (−18) | −1.8 to −4.6 (−3.0) | −2.1 to −4.0 (−3.1) | 86 to 132 (116) | +64 to +105 (+78) | 120 to 158 (148) | +30 to +86 (+60) |
| Alcohol-histamine | 15 | | | | | | | |
| alcohol phase | | 0 to −16 (−6) | +0.6 to −2.9 (−1.4) | 0 to −1.1 (−0.4) | 0 to 89 (65) | 0 to +58 (+34) | 105 to 138 (115) | +8 to +40 (+28) |
| histamine phase | | −2 to −30 (−16) | −1.5 to −3.2 (−2.5) | −2.8 to −4.6 (−3.5) | 58 to 125 (104) | +36 to +98 (+70) | 112 to 152 (139) | +22 to +80 (+54) |
| *Adrenalin | 8 | +31 to −18 (+5) | +1.1 to −2.6 (−0.9) | +0.5 to +1.2 (+0.8) | | 0 to −46 (−28) | | +18 to −46 (−7) |

*Same subjects received insulin injection.
Figures in brackets are the mean values.

TABLE V. GASTRIC JUICE AND BLOOD ELECTROLYTE ANALYSIS IN THE SAME SUBJECT: STIMULUS UNKNOWN TO SUBJECT AND INVESTIGATOR

| GASTRIC ANALYSIS | | | | | | | | BLOOD ANALYSIS | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TIME (MIN.) | VOLUME (ML.) | VOLUME (ML./MIN.) | $Na^+$ | $K^+$ | $ca^+$ | $H^+$ | $cl^-$ | TIME (MIN.) | GLUCOSE (MG. %) | $Na^+$ PL | $K^+$ PL | $cl^-$ PL | $K^+$ R.B.C. |
| | | | (MEQ./L.) | | | | | | | (MEQ./L.) | | | |
| –37 | 53* | | 61 | 8.9 | 3.20 | 37 | 117 | –57 | 86 | 150 | 4.4 | | 97 |
| – 7 | 45 | 1.5 | 50 | 8.4 | 2.95 | 53 | 114 | – 2 | 87 | 150 | 4.5 | 106 | 98 |
| *Aqua dest. intramuscularly* | | | | | | | | | | | | | |
| | | | | | | | | 12 | 89 | 152 | 4.6 | | 97 |
| 0- 38 | 25 | 0.5 | 50 | 6.9 | 3.25 | 43 | 107 | 30 | 93 | 149 | 4.8 | 104 | 97 |
| 38- 68 | 17 | 0.6 | 55 | 7.4 | | 27 | 91 | 53 | 80 | 151 | 4.5 | | 98 |
| 68- 88 | 22 | 1.1 | 53 | 7.4 | | 28 | 92 | 91 | 84 | 150 | 4.3 | 106 | 96 |
| 88-128 | 35 | 0.9 | 53 | 8.4 | 2.82 | 38 | 103 | 121 | 83 | 151 | 4.4 | | 97 |
| 128-163 | 32 | 0.9 | 61 | 7.4 | 3.08 | 26 | 103 | | | | | | |
| | | | | | | | | 183 | 87 | 150 | 4.4 | | |
| –57 | 50* | | 64 | 11.4 | 4.46 | 35 | 116 | –80 | 77 | 152 | 4.2 | 105 | 102 |
| –15 | 25 | 0.6 | 59 | 9.2 | | 50 | 118 | – 3 | 78 | 152 | 4.4 | | 100 |
| *Insulin 6 units intravenously* | | | | | | | | | | | | | |
| | | | | | | | | 10 | 69 | 153 | 4.1 | | 108 |
| 0- 40 | 70 | 1.3 | 46 | 8.3 | 2.95 | 85 | 141 | 34 | 70 | 154 | 3.8 | | 106 |
| 40- 63 | 55 | 2.4 | 67 | 5.4 | 3.40 | 64 | 136 | 59 | 46 | 154 | 3.4 | 100 | 99 |
| 63- 97 | 37 | 1.1 | 71 | 5.6 | | 53 | 131 | 92 | 77 | 156 | 4.0 | 102 | 98 |
| 97-125 | 28 | 1.0 | 72 | 5.6 | | 50 | 133 | 122 | 85 | 154 | 4.2 | | 99 |
| 125-158 | 30 | 0.9 | 74 | 5.6 | 4.10 | 43 | 131 | | | | | | |
| | | | | | | | | 182 | 87 | 156 | 4.3 | | 97 |

*Fasting sample.

The calcium content remained level or decreased slightly so that the minute excretion rose considerably by two to six times the resting amount, particularly in the early secretory phase.

ACTION OF ADRENALIN ON GASTRIC SECRETION OF ELECTROLYTES

Eight of the insulin subjects were given 0.6 to 1.0 ml. 1:1,000 Adrenalin Hydrochloride intramuscularly at a week's interval. The volumes secreted decreased, as did the total acid and total chloride contents. The gastric juice potassium suffered no change or a slight fall (+1.1 to −2.6 meq. per liter for all estimations), the sodium showed no consistent trend, while the calcium levels increased in all subjects during the test period.

The electrolyte variations for all the groups have been summarized in Table IV.

*Relation Between Hydrogen and Other Ions.*—There was a close correspondence between free acidity values and total chloride in response to secretory stimulants ($r = +0.84$, $+0.93$, and $+0.92$ for the alcohol-histamine, histamine, and insulin cases, respectively); this indicated that these specimens represented pure and uncontaminated gastric juice in most instances. The sodium, potassium, and calcium values, in general, decreased with increasing acidity. However, the correlation was not high and this is ascribed to the differential effects of histamine and insulin on the various glandular elements of the stomach to be discussed later.

Eight of the cases tested were classified as hyposecretors on the basis of fasting acidity and volume values and the response to gastric secretory stimulants. They showed higher fasting and resting values for gastric juice potassium, sodium, and calcium than normals, and lesser change in response to histamine or insulin.

*Relation to Plasma Levels.*—The gastric juice potassium was in every instance found to be greater than the corresponding plasma values. The ratio for fasting and resting specimens varied from 1.8 to 3.6:1, and was somewhat less under conditions of active secretion. No relationship between the potassium content of gastric juice and plasma was found; despite a decrease at maximal secretion of 1.4 meq. per liter of plasma potassium (mean for all subjects) after Adrenalin, there was little change in the gastric juice values, while the less marked fall in plasma potassium of 0.9 meq. per liter (mean for same subjects) after insulin injection was accompanied by a 50 per cent reduction in the gastric juice content (Table V).

Gastric juice calcium values were usually lower than normal serum calcium levels in specimens not contaminated by saliva, and exhibited considerable alterations with unaltered serum calcium levels.

Plasma sodium underwent no significant variation during active secretion, while plasma chloride showed a consistent fall at the height of secretion in all subjects (Table V).

*Mucin and Cation Content.*—Mucin was obtained by centrifugation in amounts sufficient for analysis from resting specimens, and the potassium, sodium, and calcium contents determined after drying and ashing weighed samples. Sodium values were higher than those for potassium, which were

only slightly greater than the calcium. Similar results were obtained for cation estimations made on the whole gastric juice, and a filtered specimen of the same juice and the ion concentration in the mucin calculated from the percentage volume of mucin. It was concluded that mucin contained these cation estimations, made on the whole gastric juice and a filtered specimen of the same juice, and the ion concentration in the mucin calculated from the

## COMMENT

The fundamental researches of Pavlov,[13] Hollander,[14] and Babkin[15] indicate that the composition of gastric juice is dependent upon the differential activity of the various glandular elements of the stomach in response to stimulation.

During active secretion parietal cells produce the greater volume; such secretion under normal conditions of formation is considered to be an isotonic solution (140 to 165 meq. per liter), mainly hydrochloric acid with a small amount of neutral chloride.[14, 16] The peptic and mucous glands form pepsin and mucin with a small fluid volume and a neutral chloride content in the vicinity of 100 to 120 meq. per liter. This, mixed with the parietal secretion, will neutralize the acid, the extent depending on the relative amount of the two secretions.

The response of the gastric mucosa to histamine and insulin exhibits a dissociation of effects; histamine produces an immediate temporary increase in the activity, particularly of the parietal cells, while insulin acts by hypoglycemia inducing vagal stimulation of all the glandular elements of the stomach.[15]

The volume and acidity values of histamine and insulin juice, though similar,[15, 17] cover a wide range of responses from individual to individual and in the same individual so that the differing reactions are not clearly evident. However, the pepsin content of insulin juice is several times that of histamine juice.[15, 17] Consideration of the electrolyte values presented here indicates a further differential effect. There is a virtual absence of calcium in histamine juice and a marked increase in the secretion of calcium after insulin; this occurs despite similar rates of juice secreted. Thus calcium is derived almost entirely from nonparietal sources, and the experiments on the viscous mucin indicate that the calcium of the centrifuged or filtered fluid of the gastric secretion may be derived from mucous gland secretion.

With regard to gastric juice potassium, Hollander[14] concluded that it was derived from nonparietal sources. Gray and Bucher,[18] investigating this problem in vagotomized dogs subjected to continuous histamine injections, concluded that potassium is secreted from parietal and nonparietal cells at a fairly constant rate of about 7 meq. per liter in spite of wide variations in acidity and rates of secretion. However, subsequent observations in man[19, 20] have revealed considerable variations in gastric juice potassium at the height of the secretory response to histamine and insulin, respectively.

In this series of observations gastric juice potassium values significantly above normal have been found in the fasting and resting juice of hyposecretors and achlorhydrics (unpublished). During the secretory response to insulin

and histamine there is a fall in gastric juice potassium, which is marked and prolonged in the former instance (the initial increase in gastric juice potassium in the early phase of histamine secretion may be ascribed to the "washing-out" effect of the sudden great increase in flow produced by histamine—the peptic activity of such juice shows a similar temporary increase).

The mechanism whereby hydrogen ions can accumulate in the gastric juice some $3 \times 10^6$ times that of the blood level requires considerable energy in the process of active transfer. In isolated frog gastric mucosae, the $O_2$ uptake increased 70 to 80 per cent after addition of histamine, and for a wide range of secretion rates the net amount of acid, mainly HCl, secreted on the lumen side corresponded to a chemically equivalent net amount of unidentified base given off to the vascular side.[21, 22] It has always been assumed that sodium is the chief ion affected in this interchange, but it is apparent that potassium also shows a reciprocal relationship to acid secretion.

The enhanced carbohydrate metabolism in the gastric mucosa for the energy transfer of hydrogen ions requires increased potassium uptake by its cells. This is suggested as the basis for the decreasing gastric juice potassium with increasing acidity. The differences in the effect between histamine and insulin may be due to the dissociation of the secretory response as well as the general metabolic effects of insulin on potassium. This question is at present under investigation.

Such variations in the potassium and calcium content of gastric juice, in response to histamine and insulin, lend support to the view of Glass and Boyd,[23] from their analysis of the gastric mucin and electrolyte response to insulin in man, that the nonparietal component of gastric juice, coming as it does from four different types of glands, cannot be considered to give rise to a constant "alkaline constituent" as postulated by Hunt[24] and Gray and Bucher.[18] It would therefore appear that the composition of the individual components of gastric juice may also be subject to variation, apart from the varying rate of secretion of the individual constituents of whole gastric juice.

## SUMMARY

1. The estimation of potassium, sodium, and calcium by a method of internal standard flame spectrophotometric analysis is described. Sources of interference are adequately corrected for by the incorporation of lithium as internal standard. The technique is rapid and of a statistical accuracy superior to chemical analysis and direct flame photometry.

2. Fasting and resting values of potassium, sodium, and calcium in gastric juice of 50 young healthy male adults are presented, and the variations in these cations during histamine and insulin tests of gastric function have been followed in 39 subjects.

3. The dissociated response of the different glandular elements of the stomach is evidenced from the consistent sequence of change in gastric juice potassium and calcium to histamine and insulin stimulation. The high correlation coefficient of total chloride to free acid in such specimens indicates that they can be considered as pure gastric juice in most instances. It would

appear that calcium in filtered gastric juice is derived from the nonparietal component of gastric juice, probably mucous secretion. Potassium is present both in the parietal and nonparietal components of gastric juice, and alterations during acid secretion may be related to a membrane ion-exchange of hydrogen and base and the increased potassium uptake by the gastric mucosa during active metabolism as major factors.

4. Hyposecretors have gastric juice potassium and calcium values higher than normals, and undergo less change after histamine or insulin injection.

I wish to thank Professor J. Gillman for his advice and interest in this investigation. I am also indebted to Dr. T. Gillman, who initiated the experiment on the insulin cases, and Dr. E. Rosenberg and Mr. A. van Zyl for the gastric acidity and blood glucose values in these subjects.

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# LIVER FUNCTION IN UNTREATED ADDISONIAN PERNICIOUS ANEMIA

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THE present study was undertaken to evaluate liver function in patients with untreated pernicious anemia. Previous reports of liver function tests in such patients have mainly recorded the results of single tests uncorrelated with other functional assay methods. Since the livers of patients with pernicious anemia in relapse have been shown to be deficient in the hematopoietic vitamin $B_{12}$ both by clinical[1, 2] and microbiological[3] assay, it was of interest to observe the possible effect of this deficiency state on the various hepatic cellular functions.

Although anemic anoxia is commonly offered as an explanation for hepatic dysfunction and histologic damage,[4-7] only isolated studies of tests of liver function in patients with severe degrees of anemia have appeared in the literature. If anemia is a cause of anoxia of the liver, it is apparent that our patients were subject to a considerable degree of such stress, since their erythrocyte counts ranged from 0.80 to $2.42 \times 10^6$/cu. mm. and averaged $1.56 \times 10^6$/cu. mm.

In the observations reported here, a battery of liver function tests was performed in a series of twenty unselected patients admitted to the Boston City Hospital with severe untreated Addisonian pernicious anemia.

## REVIEW OF THE LITERATURE

The relevant information reported in the literature concerning liver function tests in patients with pernicious anemia in relapse is summarized in Table I. The following statements pertain to observations not outlined there. Fouts, Helmer, and Zerfas[26] found "no marked dysfunction of the power of the liver to conjugate benzoic acid" in patients with pernicious anemia. Lindqvist and Sohrne[19] reported that oral cholecystography failed to outline the gall bladder in 16 of 33 patients with pernicious anemia in relapse; repetition of the test in 12 of these patients after remission had been induced by treatment showed definite visualization of the gall bladder in eight.

Warner and Owen[24] found the initial prothrombin concentration normal when measured by the one-stage method, but significantly low when assayed by the two-stage method. Parenterally administered vitamin K did not raise the two-stage prothrombin concentration in their patients, although elevation did occur following the injection of purified liver extract. Owren[27] stated that about 50 per cent of patients with untreated pernicious anemia have

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TABLE I. SUMMARY OF REPORTED LIVER FUNCTION STUDIES IN PERNICIOUS ANEMIA

| TEST | NUMBER OF CASES STUDIED | NUMBER NORMAL | NUMBER ABNORMAL | OBSERVER |
|---|---|---|---|---|
| Serum bilirubin level | 28 | 0 | 28 | Broun, Ames, Warren, and Peabody[8] |
| Serum bilirubin level | 9 | 7 | 2 | Vaughan and Haslewood[9] |
| Serum bilirubin level | 4 | 0 | 4 | Diamond[10] |
| Serum bilirubin level | 85 | 16* | 69 | Mills and Mawson‡[11] |
| Bilirubin clearance | 7 | 4 | 3 | Barker[12] |
| Bilirubin clearance | 5 | 1 | 4 | Harrop and Barron[13] |
| Rose bengal excretion | 5 | 1 | 4 | Pernokis and Freeland[14] |
| BSP excretion | 5 | 5 | 0 | Harrop and Barron[13] |
| BSP excretion | 1 | 1 | 0 | Serby and Bloch[15] |
| Phenoltetrachlorphthalein | 3 | 3 | 0 | Rosenthal[16] |
| Intravenous cholecystography | 22 | 13 | 9 | Layne and Boyden[17] |
| Intravenous cholecystography | 58 | 45 | 13 | Bethell and Harrington[18] |
| Oral cholecystography | 33 | 17 | 16 | Lindqvist and Sohrne[19] |
| Galactose tolerance | 4 | 1 | 3 | Wever, Althausen, et al.[20] |
| Levulose tolerance | 5 | 5 | 0 | Harrop and Barron[13] |
| Fructose tolerance | 5 | 5 | 0 | Greene and Connor[21] |
| Alkaline phosphatase | 6 | 6 | 0 | Gutman[22] |
| Colloidal gold | 8 | 3 | 5 | Maclagan[23] |
| Thymol turbidity | 8 | 5 | 3 | Maclagan[23] |
| Prothrombin (two-stage) | 20 | 2† | 18 | Warner and Owen[24] |
| Hippuric acid excretion | 3 | 0 | 3 | Henderson and Splatt[25] |
| Oral hippuric acid excretion | 3 | 1 | 2 | Barker[12] |

*Upper limit of normal considered by present authors to be normal mean +2 SD.

†Concentrations of 95 per cent ±25 were considered normal by the present authors.

‡In 85 untreated cases of pernicious anemia Mills and Mawson found a mean total serum bilirubin of 0.98 mg./100 ml., with a standard deviation of ±0.06. In 53 of their cases studied after treatment, the mean total serum bilirubin was 0.31 mg./100 ml., with a standard deviation of ±0.03. For eighty-five normal subjects the mean and standard deviation was 0.31 mg./100 ml. ±0.02.

prothrombin levels of about 60 to 80 per cent of normal (demonstrated by special one-stage procedures) that do not respond to vitamin K, purified liver extracts, vitamin $B_{12}$, or folic acid. The low prothrombin values returned to normal after treatment with crude liver extracts or whole liver. The required substance in liver has no antianemic effect, and has provisionally been called the protein synthesis (P.S.) factor. Ungley[28] stated that prothrombin values estimated by the two-stage method[29] have usually been normal in pernicious anemia patients in relapse and have always been normal in remission.

In 1887 Hunter[30] noted that one constant pathologic finding in pernicious anemia was a large excess of blood pigment within the liver. Mettier[31] examined the liver tissue of 20 patients with untreated pernicious anemia who died of intercurrent diseases and of 5 patients who died of intercurrent diseases while in various stages of response to liver therapy. In the untreated patients marked hypertrophy and desquamation of the Kupffer's cells were noted. Siderosis was marked in the liver cells and moderate in the Kupffer's cells. Following antipernicious anemia therapy the Kupffer's cells returned to normal size and few then contained pigment. The return to normal size took place in ten days, and the degree of desquamation of Kupffer's cells and phagocytosis of erythrocytes was estimated to be equivalent to the stage of relapse. Only a slight degree of siderosis was found in patients who died with normal blood levels. Finch[32] observed an excessive deposition of iron in the parenchymal and reticuloendothelial tissue of the liver in 21 of 24 patients who died of severe, untreated pernicious anemia. In contrast to this, in only 2 of 11 patients with pernicious anemia under adequate treatment at the time of death were significant amounts of hemosiderin found in the liver.

TABLE II. LIVER FUNCTION TESTS IN

| | CASE | AGE (YR.) | SEX | R.B.C. × $10^6$/CU. MM. | HEMATOCRIT (%) | BSP % RETENTION AT 45 MIN. | BILIRUBIN TOTAL MG./100 ML. | BILIRUBIN 1 MIN. REACTING MG./100 ML. (DIRECT) | ICTERUS INDEX UNITS |
|---|---|---|---|---|---|---|---|---|---|
| Normal values | | | M | 5.4 ± 0.8 | 47 ± 7 | < 5 | 0.62 ± 0.25* | 0.11 ± 0.05† | 4-7 |
| | | | F | 4.8 ± 0.6 | 42 ± 5 | | | | |
| Addisonian pernicious anemia | 1 | 78 | F | **1.70‡** | **21.1** | 1.5 | 0.35 | 0.19 | 5 |
| | 2 | 69 | F | **1.59** | **20.2** | 0.0 | 0.18 | 0.06 | 5 |
| | 3 | 62 | F | **0.95** | **10.1** | 4.3 | 1.24 | 0.22 | 20 |
| | 4 | 68 | F | **1.89** | **20.0** | **7.6** | 0.70 | 0.22 | 5 |
| | 5 | 74 | F | **1.77** | **21.4** | 4.4 | 0.00 | 0.01 | 5 |
| | 6 | 68 | F | **0.90** | **11.3** | 4.0 | **2.20** | **0.55** | 10 |
| | 7 | 73 | M | **1.48** | **19.1** | **20.7** | 1.50 | **0.35** | 5 |
| | 8 | 68 | F | **1.65** | **22.7** | 3.7 | 1.03 | 0.15 | 5 |
| | 9 | 75 | F | **1.46** | **21.8** | 3.3 | 0.50 | 0.16 | 3 |
| | 10 | 70 | F | **0.80** | **10.5** | 2.2 | 1.48 | **0.69** | 20 |
| | 11 | 80 | F | **2.42** | **30.0** | 4.1 | 0.03 | 0.02 | 5 |
| | 12 | 75 | M | **1.98** | **25.3** | 1.5 | 0.67 | 0.24 | 4 |
| | 13 | 50 | M | **2.08** | **22.5** | **13.4** | 1.24 | **0.37** | 12 |
| | 13A | 50 | M | **1.73** | **19.3** | 1.6 | 0.87 | 0.22 | 7 |
| | 14 | 83 | M | **1.30** | **17.6** | 3.19 | 0.50 | 0.09 | 3 |
| | 15 | 69 | M | **1.65** | **18.8** | 2.5 | 0.60 | **0.37** | 7 |
| | 16 | 51 | M | **1.41** | **17.1** | 1.8 | 0.13 | 0.11 | 5 |
| | 17 | 62 | M | **1.29** | **16.5** | 1.5 | 1.46 | **0.35** | 12 |
| | 18 | 44 | M | **1.58** | **21.0** | 2.29 | 0.80 | 0.14 | 5 |
| | 19 | 78 | M | **1.30** | **16.6** | | 1.00 | **0.45** | 10 |
| | 20 | 73 | F | **1.91** | **20.3** | 4.0 | 1.00 | **0.27** | 7 |
| Nutritional megaloblastic anemias | 21 | 29 | F | **2.79** | **24.9** | 1.0 | 0.64 | 0.22 | 5 |
| | 22 | 43 | F | **1.50** | **18.5** | **8.0** | 0.91 | 0.03 | 4 |
| | 23 | 43 | F | **1.43** | **16.9** | **14.4** | 1.20 | **0.90** | 7 |
| | 24 | 31 | M | **1.33** | **13.8** | 2.55 | 0.54 | 0.24 | 5 |
| | 25 | 42 | M | **1.12** | **11.5** | **16.5** | **2.02** | **0.35** | 10 |
| | 26 | 35 | F | **0.83** | **10.5** | 0 | 0.34 | **0.28** | 3 |

*Upper limit of normal considered to be 1.5 mg./100 ml.[30]
†Upper limit of normal considered to be 0.25 mg./100 ml.[30]
‡Abnormal values in boldface type.

In a study of patients dying with pernicious anemia in relapse, Rich[4] noted atrophy of the liver cells adjacent to the efferent vein. Correlating further necropsy studies and some experimental observations, he concluded that anoxia was the cause of the atrophy. Such atrophy has not been noted consistently by other observers.

## METHODS

Observations were made on 20 patients with Addisonian pernicious anemia (Cases 1 through 20, Table II) diagnosed by characteristic peripheral blood and bone marrow morphology, histamine-fast achlorhydria, and subsequent response to antipernicious anemia therapy.* Studies were made on Case 13 in two different relapse periods.

Observations were also made on six additional patients (Cases 21 to 26, Table II) with severe macrocytic anemias and megaloblastic bone marrows. Case 21 had megaloblastic anemia of pregnancy with histamine-resistant achlorhydria. A satisfactory hematologic response was not made until folic acid was administered. Cases 22 through 25 had free hydrochloric acid in their gastric contents following histamine stimulation and extremely poor dietary intakes for long periods before entry into the hospital. They were considered to have nutritional megaloblastic anemia. Case 26 was a markedly undernourished patient with diarrhea, cheilosis, and severe glossitis. Because of these findings she was also considered to have nutritional macrocytic anemia, although there was no hydrochloric acid in

UNTREATED PERNICIOUS ANEMIA

| CEPHALIN FLOCCULATION 48 HR. | THYMOL TURBIDITY ML. $BaSO_4$ | THYMOL FLOCCULATION | ALKALINE PHOSPHATASE (UNITS) | PROTHROMBIN CONCENTRATION (% NORMAL) | TOTAL PROTEIN (GM./100 ML.) | ALBUMIN (GM./100 ML.) | GLOBULIN (GM./100 ML.) |
|---|---|---|---|---|---|---|---|
| 0–2+ | < 1.7 ml. | 0 | 2.0 – 4.5 | 95 ± 25 | 6 – 7 | 4 – 5 | |
| 1+ | 0.1 | 0 | 3.0 | 100 | 4.47 | 2.00 | 2.47 |
| 0 | 1.8 | 0 | 3.1 | 105 | 4.42 | 2.10 | 2.32 |
| 0 | 1.2 | 0 | 2.3 | 50 | 5.02 | 4.38 | 0.64 |
| 1+ | 0.5 | 0 | 3.0 | 43 | 5.10 | 3.07 | 1.93 |
| 1+ | 1.5 | 0 | 2.2 | 50 | 6.79 | 3.88 | 3.07 |
| 2+ | 1.6 | 0 | 2.5 | 125 | 5.30 | 3.18 | 2.12 |
| 1+ | 1.6 | 0 | 2.5 | 70 | 6.17 | 3.75 | 2.42 |
| 1+ | 1.0 | 0 | 2.2 | 75 | 6.13 | 4.07 | 2.06 |
| 1+ | 2.6 | 0 | 2.3 | 80 | 4.94 | 3.01 | 1.93 |
| 1+ | 2.5 | 0 | 2.4 | 60 | 6.47 | 4.59 | 1.88 |
| 1+ | 2.1 | 3+ | 3.7 | 112 | 5.23 | 4.57 | 0.66 |
| 1+ | 1.9 | 0 | 2.2 | 99 | 5.62 | 4.14 | 1.48 |
| | 2.8 | 0 | | 145 | 6.62 | 4.60 | 2.02 |
| 2+ | 2.3 | | 4.0 | 80 | 6.93 | 4.52 | 2.41 |
| 1+ | 2.2 | 0 | 4.4 | 72 | 5.28 | 3.13 | 2.15 |
| 2+ | 2.7 | 1+ | 4.8 | | 6.57 | 4.78 | 1.73 |
| 0 | 0.8 | 0 | 1.2 | 70 | 5.77 | 2.73 | 3.04 |
| 0 | 0.7 | 0 | 2.7 | | 5.14 | 3.52 | 1.62 |
| 1+ | | | 1.7 | 115 | 5.54 | 3.28 | 2.26 |
| 3+ | | | 3.2 | 95 | 6.26 | 3.99 | 2.27 |
| 1+ | 2.0 | 0 | 1.5 | 85 | | | |
| 0 | 4.3 | 0 | 2.5 | 100 | 5.7 | 4.20 | 1.50 |
| 1+ | 2.2 | 0 | 3.5 | 120 | 6.06 | 4.56 | 1.50 |
| 0 | 2.1 | 0 | 4.1 | 120 | 7.34 | 3.81 | 3.53 |
| 2+ | 1.2 | 0 | 2.1 | 63 | 5.93 | 3.80 | 2.04 |
| 1+ | 0.8 | 0 | 5.3 | 63 | 6.52 | 4.04 | 2.48 |
| 2+ | 1.4 | 0 | | 100 | 5.50 | 3.30 | 2.20 |

the gastric juice after histamine. None of this group had evidence of subacute combined degeneration of the spinal cord. It is of interest to note that the average age of the 20 patients with pernicious anemia was 69 years, as contrasted to an average age of 37 years for the others.

To avoid complicating the results and discussion of the findings in pernicious anemia, no further mention will be made of this heterogeneous group in which the specific deficiency responsible for the anemia was not established. It would seem sufficient to point out that there are no significant differences between the patients with pernicious anemia and those with the nutritional megaloblastic anemias as regards liver function tests.

The liver function tests employed were icterus index, serum bilirubin concentration,[33, 34] thymol turbidity and thymol flocculation,[23, 35] plasma prothrombin concentration,[36] cephalin flocculation,[37] bromsulfalein (BSP) retention,[38] serum total protein and albumin concentration,[39] and serum alkaline phosphatase activity.[40]

## RESULTS AND DISCUSSION

The results of the above tests and their normal values are presented in Table II.

The BSP retention exceeded 5 per cent in 3 of the 20 patients with pernicious anemia. In 2 of these patients (Cases 7 and 13) showing definitely ab-

*Patients 10 and 20 died before response to therapy could be evaluated.

normal BSP retention (20.7 and 13.4 per cent, respectively), there were clear-cut histories of excessive ingestion of alcohol and of grossly inadequate food intake over prolonged periods. The subsequent hematologic relapse of Case 13 clearly suggests the relevance of these factors, rather than the anemia, to the liver dysfunction. Thus, after discharge from the hospital in excellent remission, the patient did not return for further therapy until eight months later when he was readmitted (as Case 13A) with severe anemia. He then reported that since his last hospitalization he had been eating well and had been abstaining from alcohol. The BSP retention was normal (1.6 per cent), even though the anemia was more severe than on the previous admission (see Table II). In the other patient (Case 4) demonstrating an abnormal BSP test (7.6 per cent retention) there was no history of a grossly inadequate diet nor of the ingestion of alcohol, but there was evidence of cardiac decompensation probably sufficient to account for the mild degree of dye retention.[41] Zamcheck and associates[42] have demonstrated that patients with severe anemia due to recent blood loss may have a normal BSP retention. In the patients with anemia reported here, there was also no apparent correlation between BSP retention and degree of anemia.

Zieve and associates[34] have defined the normal variations in the one-minute and total serum bilirubin concentrations on the basis of 719 determinations. The mean normal one-minute bilirubin was found to be 0.11 ± 0.05 mg./100 ml., and the upper limit of normal was considered to be 0.25 mg./100 ml.; the mean total bilirubin, 0.62 ± 0.25 mg./100 ml., and the upper limit of normal 1.5 mg./100 ml. Accepting 1.5 mg./100 ml. as the upper limit of normal for total serum bilirubin[34] only one untreated patient (Case 6) showed an abnormally increased value of 2.20 mg./100 ml. Eight patients exceeded the upper limit of normal allowed for the one-minute bilirubin level, but these elevations were for the most part small. The mean values and the standard deviations for serum bilirubin were calculated separately for the 20 patients with untreated pernicious anemia and for 43 other patients with pernicious anemia under adequate treatment. The results are shown in Table III.

TABLE III. SERUM BILIRUBIN* VALUES IN TREATED AND UNTREATED PERNICIOUS ANEMIA CASES

| | NO. CASES | TOTAL | | ONE MINUTE | | INDIRECT | |
|---|---|---|---|---|---|---|---|
| | | MEAN | S.D.† | MEAN | S.D.† | MEAN | S.D.† |
| Untreated pernicious anemia | 20 | 0.83 | ±0.42 | 0.25 | ±0.17 | 0.58 | ±0.44 |
| Treated pernicious anemia | 43 | 0.51 | ±0.28 | 0.13 | ±0.09 | 0.38 | ±0.29 |
| Controls | 34 | 0.62 | ±0.25 | 0.11 | ±0.05 | - - - | - - - |

*As mg./100 ml. serum.
†Standard deviation.

The one-stage prothrombin concentration was less than 70 per cent in four of nineteen patients; only one of these was below 50 per cent.

The serum total protein concentrations were lower than the accepted range for the method used in 12 of the 20 patients studied. In 10 of these cases the albumin concentrations were also low. At best the method used is only moderately accurate and, in view of the advanced age and poor economic status

of these patients, the abnormal protein values may well reflect the dietary deficiencies of chronically ill patients rather than disordered liver function. Significant protein abnormalities were not demonstrated by the turbidity and flocculation tests.

The data presented in Table I demonstrate that, although abnormal liver function tests in this series of patients are fairly frequent, there is no consistent pattern of abnormality reproduced from patient to patient. It should be pointed out that the average age of the patients wih pernicious anemia was 69 years. Rafsky and Newman[43] evaluated liver function in 50 healthy individuals aged 63 to 93 years by means of cephalin flocculation, BSP excretion, cholesterol levels, and hippuric acid excretion tests. At least one abnormal test was found in 86 per cent of these patients. The frequency of abnormal results in our series of seriously ill patients does not then seem unusual.

The generally accepted concept of hyperbilirubinemia in pernicious anemia must have arisen at a time when the hemolytic component of this deficiency disease played a more active part than it does now. Before adequate therapy was known, the outcome of the disease was almost invariably fatal in one to three years (44) and the course was progressive although temporarily interrupted by one (85 per cent) or more (15 per cent) remissions of varying duration and benefit. It would seem probable that during the latter part of this course the deficiency state would be more absolute in nature and the hemolytic manifestations more marked, resulting in hyperbilirubinemia. The possibility exists, of course, that with a more prolonged severe deficiency, liver function would be significantly altered. It is apparent that clinical jaundice or hyperbilirubinemia is not necessary for the diagnosis of Addisonian pernicious anemia. Examination of the statistical analysis of the total and indirect bilirubin values shows that, although the mean values for the untreated patients are greater than those for the treated patients (and the accepted normal figures), the differences are small and there is considerable overlapping of the values in the two groups within the standard deviation range.

Since it has been demonstrated that extracts of livers of patients who die with untreated pernicious anemia contain no hematopoietic substances by clinical assay[1, 2] and no vitamin $B_{12}$ activity by microbiological assay,[3] these tissues are undoubtedly deficient in vitamin $B_{12}$. It is apparent then that in the present series of patients with pernicious anemia the deficiency of vitamin $B_{12}$ did not regularly disturb the integrity of hepatic cellular functions assayed by the liver function tests employed.

The low-oxygen carrying power of anemic blood and the low hepatic venous blood oxygen saturation (41 per cent) are presumed to effect a considerable anoxic stress on the liver parenchymal cells despite an increased hepatic blood flow in markedly anemic subjects.[49] On the basis of this mechanism various authors have attempted to explain the centrolobular hepatic necrosis seen in severe anemia.[4, 5, 6, 7, 45] However, in the present series of severely anemic patients, there is no evidence that such an anoxic stress produced any consistent abnormalities of liver function demonstrable by the assay methods used.

## SUMMARY AND CONCLUSIONS

A group of liver function tests was performed on twenty patients with untreated Addisonian pernicious anemia. No evidence was obtained for a regular pattern of hepatic dysfunction. Vitamin $B_{12}$ deficiency marked enough to cause severe pernicious anemia does not regularly impair the liver function as measured by the tests employed.

Total serum bilirubin exceeded the normal limits in only one of the patients and clinical jaundice was noted in only three. Absence of jaundice or hyperbilirubinemia is not evidence against the diagnosis of pernicious anemia.

If anemic anoxia of the liver cells occurs in severe pernicious anemia, it does not regularly impair hepatic function as measured by the tests employed.

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# A STEREOVECTORCARDIOGRAPH

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AFTER Einthoven first demonstrated the principles of current distribution around the heart as applied to the electrocardiogram, it has been possible to construct uniplane vectorcardiograms from any two electrocardiographic leads. Also, by appropriate combination of three or more leads, it has been possible to construct third dimensional vectorcardiograms. In order to facilitate study of vectorcardiograms, instruments utilizing either optical or cathode-ray oscillographs were first developed some fifteen years ago to make uniplane recordings directly.[1, 2] These are now used to a very great extent, but, in order to study the third dimensional vectorcardiogram, it has still been necessary to record the vectorcardiogram in at least two planes and then construct a third dimensional vectorcardiogram in the form of a wire model.

For the past year and one-half, this department has been engaged in developing a stereovectorcardiograph for instantaneous registration of the third dimensional vectorcardiogram. Concurrently, Schmitt and co-workers have been working on a similar device, and preliminary results of their work have been reported recently.[3] The Schmitt apparatus utilizes two separate oscilloscopic screens, and two vectorcardiograms are recorded simultaneously from planes of the body which deviate a few degrees from each other. One of these images is recorded on one of the oscilloscopic screens, and the other on the second oscilloscopic screen. By appropriate photographic and optical systems, one of these images is reflected into one eye of the observer and the other into the opposite eye, so that a third dimensional effect is attained.

The apparatus which has been designed in this laboratory utilizes only one cathode-ray screen, and the over-all apparatus has been simplified to the point that clinicians can make stereovectorcardiograms which depict third dimensional vectorcardiograms almost as easily as he now makes the uniplane recordings.

## THE STEREOVECTORCARDIOGRAPHIC APPARATUS

Fig. 1 illustrates in a block diagram the theory of the stereovectorcardiograph. It will be noted that this apparatus utilizes a 5-inch cathode-ray tube which has two separate beams, one of these beams playing on the left side of the screen and the other on the right side of the screen. It will be noted that the cardiographic impulses picked up by electrodes *A*, *B*, *C*, and *D* control the electron beam passing between the deflection plates *1*, *2*, *3*, and *4*. Such control is effected by appropriate amplification through the vertical amplifier and one of the horizontal amplifiers. Consequently, vectorcardiogram I is recorded from the plane designated by the points *A*, *B*, *C*, and *D*. Similarly, vectorcardiogram II is recorded

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from plane *A, B, C, E*, utilizing the vertical amplifier and the other horizontal amplifier and with the electron beam being controlled this time by deflection plates *5, 6, 7,* and *8*. Thus, two vectorcardiograms are recorded simultaneously on the oscilloscopic screen from two separate planes which are angled to each other at approximately 10 to 15 degrees. It will be noted that plane *A, B, C, D* is approximately perpendicular to the line of sight when an observer stands directly in front of the patient some 12 inches away and looks at him with his left eye. On the other hand, plane *A, B, C, E* is approximately perpendicular to the line of sight of the right eye under the same condition. Therefore, when the observer views vectorcardiogram I with his left eye and vectorcardiogram II with his right eye and fuses these images appropriately, he gains the third dimensional effect of the vectorcardiogram.

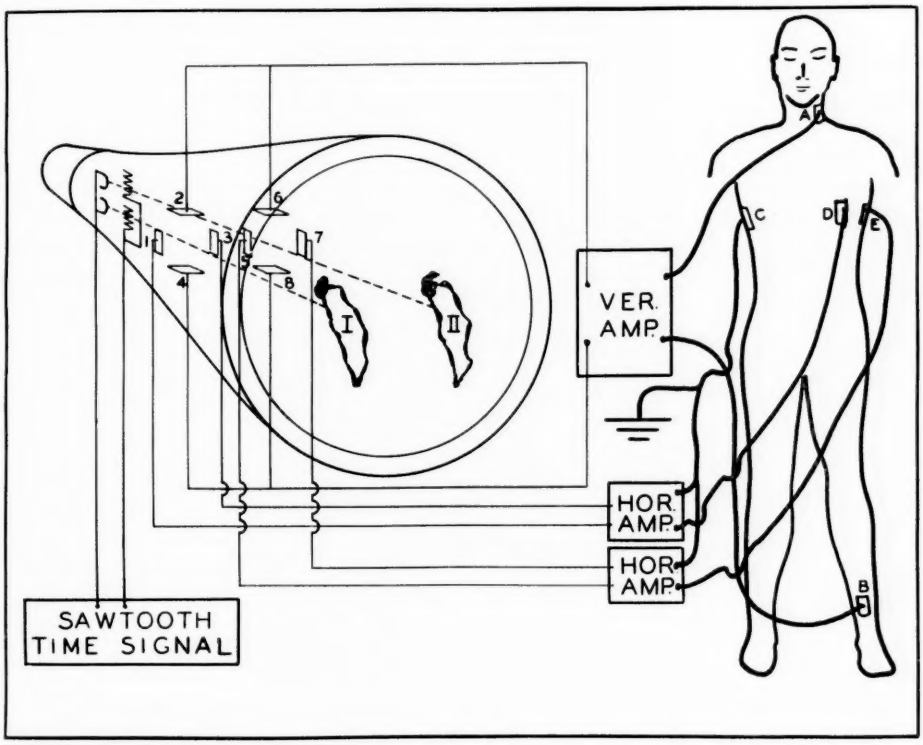


Fig. 1.—Block diagram of the stereovectorcardiograph (explained in the text).

In order to have a time signal on the vectorcardiogram, and also in order to determine which direction the impulse travels, a saw-tooth signal has been applied to the Z axis of both beams simultaneously. This saw-tooth time signal may be operated at any frequency, but a frequency of approximately 300 cycles per second gives approximately the best results. Because the saw-tooth time signal turns the beam on slowly and then cuts it off rapidly, this gives each pip of the vectorcardiogram a comet-like appearance, and the impulse travels toward the head of the comet.

Viewing of the stereovectorcardiogram to obtain the third-dimensional effect can be accomplished either by direct observation of the images on the oscilloscopic screen or by photographing the images first. When direct observation is employed, one must wear glasses with approximately plus 7 diopters strength in order to attain best fusion. In this manner, the third dimensional vectorcardiogram can be viewed for each beat of the heart. On the other hand, photographs may be made for single heart cycles with an os-

cillographic camera. In this laboratory a Polaroid-Land camera is used so that the photographs can be developed and a positive print made in one minute's time. The images obtained on the photographs are smaller in size than are the direct images, and usually can be fused with the naked eyes.

Fig. 2 illustrates actual stereovectorcardiograms which have been recorded from a normal individual. The upper record was recorded while using the saw-tooth time signal to indicate the direction in which the impulse was travelling, and the lower recording was made without the time signal. After a little practice the images as recorded in this particular picture can be fused with the naked eyes without the benefit of special optical

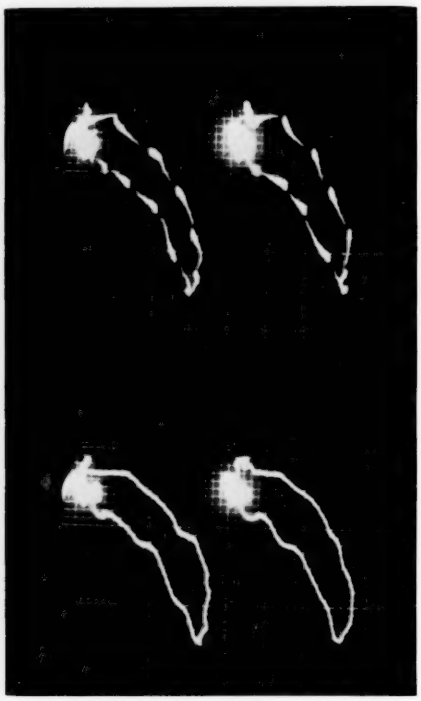

Fig. 2.—Stereovectorcardiograms recorded from a normal individual. The above pair is recorded with the time intervals of 1/300 of a second superimposed. The impulse travels toward the head of the cometlike pips. One millivolt is equal to 10 of the small calibration lines. These records are actual size as recorded by the oscilloscopic camera, and the third dimensional vectorcardiogram can be observed by fusing each pair of images, using the naked eyes, at a distance of 6 inches.

apparatus. Such fusion, however, is not very easy for an older individual with considerable degree of presbyopia. In these individuals corrective lenses of plus 3 to 5 diopters may be needed in order for clear fusion to take place. The third dimensional effect can be noted with greatest detail when these images are held approximately six inches in front of the eyes, and, though it may require ten to fifteen minutes practice on the part of the beginning observer, one soon learns to fuse the images immediately and rapidly so that a third dimensional vectorcardiogram can be seen. When the images are fused, one actually sees a central third dimensional vectorcardiogram which represents the fused images, and he also sees a uniplane vectorcardiogram out to each side. These separate uniplane vectorcardiograms can be blocked out by placing a barrier of cardboard between the two vectorcardiograms before attempting to fuse them. However, this has been found to be totally unnecessary after one becomes accustomed to this procedure.

Technically, the dual beam oscilloscope that was used in these studies was a Dumont Type 322. Three low-frequency amplifiers of several different designs, the designs being unimportant, have been utilized as the vertical amplifier and two horizontal amplifiers. A 1 millivolt calibration signal has been available in each of these. The saw-tooth time signal was derived from a separate gas discharge oscillator.

Placement of electrodes *A*, *B*, and *C* is not very critical, but slight shifting of electrodes *D* and *E* can change the vectorcardiograms tremendously. As a standard procedure in this laboratory, electrode *D* is placed directly over the apex of the heart, and electrode *E* is placed in the midaxillary line.

A second method of recording the stereovectorcardiogram has been used in this laboratory, utilizing a single-beam oscilloscopic tube. This second method employs an electronic switch between the two horizontal amplifiers and the oscilloscope so that the electron beam is split every $\frac{1}{2,000}$ of a second, generating first a portion of vectorcardiogram I, then a portion of vectorcardiogram II, then once again another portion of vectorcardiogram I, etc.


## SUMMARY

A stereovectorcardiograph utilizing a dual-beam oscillograph has been described, and this apparatus is almost as simple as is the uniplane vectorcardiograph. Two vectorcardiograms are recorded simultaneously so that the planes of these deviate from each other by approximately 10 to 15 degrees. When the resultant vectorcardiograms are viewed stereoscopically by the two eyes, a third dimensional vectorcardiogram is observed. This third dimensional effect can be attained by direct viewing of the oscilloscopic screen through an appropriate optical system or by viewing the recorded photograph with the naked eyes.


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# EFFECTS OF P-(DI-N-PROPYLSULFAMYL) BENZOIC ACID (BENEMID)* ON FREE AND TOTAL SULFONAMIDE PLASMA LEVELS

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## INTRODUCTION

P(DI-N-PROPYLSULFAMYL) BENZOIC ACID (Benemid) enhances penicillin concentrations[1-4] and promotes the urinary excretion of both uric acid[5, 6] and phosphates.[7] Such activity probably resides in its ability to produce a reversible inhibition of one or more renal tubular enzyme transport systems.

Sulfonamides are handled by tubular exchange mechanisms[8] and are structurally similar to certain compounds whose renal elimination is effected by Benemid. Since sulfonamides are commonly administered in conjunction with penicillin, and since Benemid is frequently used concomitantly with the latter agent in order to enhance the penicillemia,† it may be anticipated that penicillin, sulfonamides, and Benemid will be administered concurrently. Accordingly, it seemed desirable to test the effects of Benemid on sulfonamide plasma concentrations. This communication reports the results of such a study on 12 human subjects.

## MATERIALS AND METHODS

This investigation consisted of two studies. The first was an attempt to estimate, in 6 patients, the effects of orally administered Benemid on sulfonamide plasma concentrations resulting from a single intravenous injection of a triple sulfonamide‡ preparation. The second part of the study was performed on an additional 6 patients for the purpose of determining the effects of Benemid on plasma concentrations of sulfonamides following the repeated oral administration of a triple sulfonamide-penicillin preparation.

All patients were free of renal disease, as judged by a normal urinalysis and blood urea nitrogen, and each patient served as his own control.

A cross-over type of experimental design was employed in carrying out the two phases of both Part I and Part II of this investigation. Thus, during the first phase of Part II, 3 of the 6 patients received the triple sulfonamide-penicillin preparation plus Benemid,† while the remaining 3 received only the triple sulfonamide-penicillin combination. During the second phase, the medications were reversed. Each phase was separated by a period of three to six days in order that the drug, or drugs, employed might be eliminated from the body before beginning the next phase.

The dosages of the respective preparations and the time of blood sampling was as follows:

*Part I.*—In those patients receiving Benemid, this agent was administered in a dosage of 0.5 Gm. every six hours, commencing twenty-four hours prior to the sulfonamide injection and continuing for forty-eight hours thereafter. Three grams of the triple sulfonamide preparation‡ were administered intravenously to each of the six subjects in each phase of the study.

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*Benemid is Sharp & Dohme, Inc.'s trademark for p-(di-n-propylsulfamyl) benzoic acid, the generic name of which is probenecid.

†Penicillemia is a synonym that has been coined for the more cumbersome phrase "penicillin plasma concentrations" or "concentrations of the penicillin in the plasma."

‡Neotresamide sterile solution 6 per cent (Sharp & Dohme, Inc.).

| | |
|---|---|
| Sodium sulfamerazine | 0.6 Gm. |
| Sodium sulfadiazine | 1.2 Gm. |
| Sodium sulfamethazine | 1.2 Gm. |

A blank blood was drawn in all cases, prior to the beginning of each phase, and subsequent blood specimens were drawn at two, four, six, eight, twenty-four, and forty-eight hours after the injection of the triple sulfonamide preparation.

*Part II.*—Each phase covered a period of five days. Penicillin-triple sulfonamide-Benemid* tablets and penicillin-triple sulfonamide tablets† were used. As noted below, the amounts of penicillin and triple sulfonamides in each preparation were identical. Four tablets of the respective medications were administered by mouth on the first morning of each phase. This was followed by two tablets four times a day for five days. Tablets were ingested approximately one-half hour before meals and at bedtime. A blank blood was drawn in each case just prior to the initial dose, and then every day, four hours after the first daily dose.

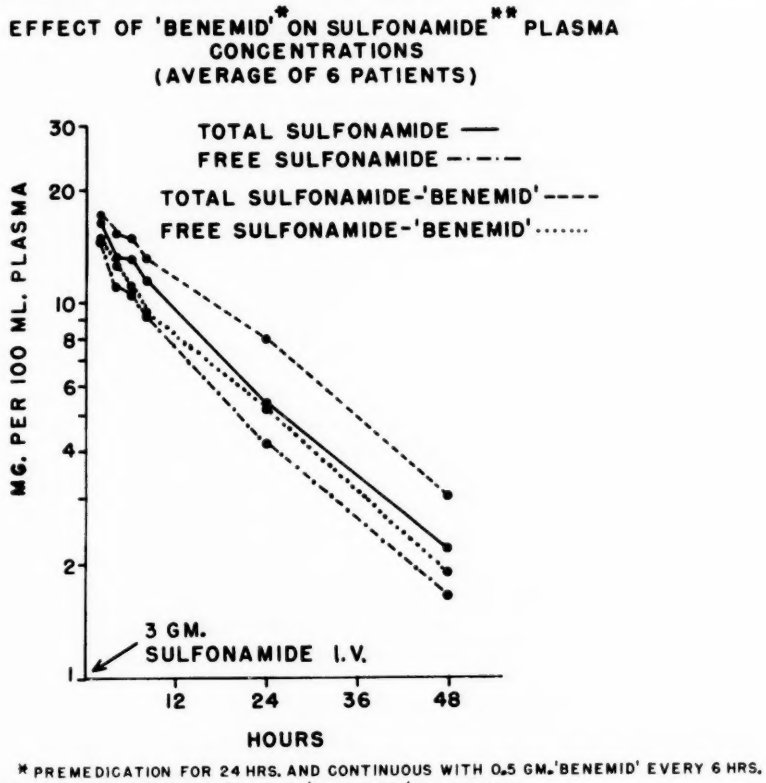


Fig. 1.

Penicillin assays were performed according to the Sarcina lutea cup-plate method.[9] Free and total sulfonamide determinations were carried out by modifications of the Bratton-Marshall method[10] and reported as milligrams of sulfamerazine per 100 ml. of plasma. It should be noted that Benemid does not interfere with sulfonamide determinations and gives no color reaction even after the acid hydrolysis employed for the determination of conjugated sulfonamides.

*Benemid with pentresamide
Each tablet = Benemid 0.25 Gm.
Potassium Penicillin G 100,000 units
Sulfamerazine ----------0.1 Gm.
Sulfadiazine ----------0.2 Gm.
Sulfamethazine ----------0.2 Gm.
The authors are indebted to Mr. Paul W. Wilcox of Sharp & Dohme, Inc., Philadelphia, Pa., for preparing this formula for investigational use.

†Pentresamide (Sharp & Dohme, Inc.)
Each Tablet = Potassium Penicillin G 100,000 units
Sulfamerazine ----------0.1 Gm.
Sulfadiazine ----------0.2 Gm.
Sulfamethazine ----------0.2 Gm.

RESULTS

Results of both parts of the study are summarized in Figs. 1 and 2.

Part I demonstrated no statistically significant difference in free sulfonamide plasma concentrations at any point throughout the test (average increase = 0.55 mg. per 100 ml.). However, with the exception of the two and forty-eight hour specimens, the total sulfonamide levels were significantly increased (average increase = 1.4 mg./100 ml., $p = < 0.001$). The increase in total sulfonamide concentrations was due to a significant increase in the plasma concentrations of the acetylated fraction (average increase = 0.89 mg./100 ml., $p = < 0.05$).

Part II again demonstrated the ability of Benemid to produce a highly significant increase in the total sulfonamide levels (average increase = 4.6 mg./100 ml., $p = < 0.001$). Unlike Part I, however, free sulfonamide plasma levels were significantly increased following continuous oral administration

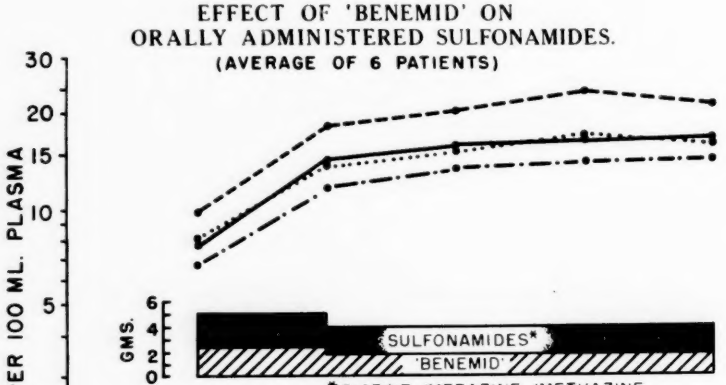


Fig. 2.

(average increase = 2.2 mg./100 ml., $p = < 0.001$). There was no evidence that the increased sulfonamide plasma concentrations, as the result of Benemid administration, were associated with a cumulative phenomenon. The plasma concentrations of sulfonamides observed following the dosage used in this study were well within the therapeutic range for the treatment of infections due to sulfonamide-susceptible organisms.

As might be expected in the presence of Benemid, the penicillin levels were significantly increased (average increase = 0.18 unit/ml., $p = <0.001$), but the degree of enhancement was not as great as ordinarily observed.[1-4]

## DISCUSSION

The results, reported above, indicate the ability of Benemid to increase total sulfonamide plasma concentrations when a triple sulfonamide preparation is repeatedly administered by mouth, or injected intravenously as a single dose. Free sulfonamide levels were significantly enhanced when sulfonamides were repeatedly administered by mouth, but not when the single parenteral dose of sulfonamides was given.

There is no ready explanation for the enhancement of the free sulfonamide plasma levels. The three forms of sulfonamides employed are said to be eliminated in the kidney by glomerular filtration and tubular reabsorption.[8] Considering the observed results a number of speculations come to mind. There is the possibility that Benemid displaces sulfonamides bound by blood proteins, and that this might transiently elevate the free sulfonamide concentration. Benemid could possibly decrease the rate of acetylation, but this is unlikely in view of the observed parallelism in the elevation of free and total sulfonamide levels. The lack of effect of Benemid on the rate of glomerular filtration has been demonstrated.[5, 13] One might postulate, therefore, an enhancement of the rate of tubular reabsorption of the free form or theorize about a three-phase renal transport of sulfonamides, such as described by Barclay, Cooke, and Kenney for potassium and phosphates.[11] Should this latter situation obtain, Benemid might be inhibiting the secretion of free sulfonamides. To suppose that Benemid acts by enhancing tubular reabsorption of sulfonamides is to suggest a confusing combination of tubular effects, i.e., the enhancement of tubular reabsorption of sulfonamides while depressing the tubular reabsorption of urates and phosphates, and inhibiting the tubular secretion of such substances as penicillin. The possibility that free sulfonamides are handled by a tubular secretory transport system that is inhibited by the action of Benemid receives little support from studies of renal clearances of sulfonamides. The clearances of free sulfonamides used in this investigation are less than that of inulin;[8] the sulfonamide/inulin clearance ratio is not altered in patients with renal disease,[8] and studies on the excretion of sulfamerazine in the dog by Earle[12] would seem to indicate that there is no evidence of a Tm phenomenon, even though the plasma concentrations were altered over a range of from 2 to 43 mg. per 100 milliliter. If a three-phase system does exist in human beings for the excretion of free sulfonamides, it seems likely that it will be demonstrated only by a study such as this which would interfere with the secretory component, since the toxicity of the sulfonamides would preclude doses high enough to reach a Tm.

The rise in the total sulfonamide plasma concentrations may be more readily supported by the literature because there is evidence that acetyl sulfamerazine, and to some extent acetyl sulfadiazine, are filtered and secreted by the renal tubules.[8] Hence, one might postulate that the observed rises in total sulfonamide concentrations are due to the ability of Benemid to produce an inhibition of certain tubular secretory enzyme transport mechanisms in a manner similar to that observed with substances such as sodium para-aminohippurate, phenol red, and penicillin.[13]

The final explanation of these results depends on renal clearance studies, designed to test the effects of Benemid on the free and acetylated forms of the individual sulfonamide drugs used in this study. Such studies are currently in progress.

From the practical standpoint, certain points deserve mention. The dosage schedule of triple sulfonamides used in this study conforms to that currently recommended. Triple sulfonamides were chosen for this study because of their wide current usage and because of the paucity of data on plasma concentrations following their use. If one assumes that the therapeutic concentrations of these agents are between 8 and 10 mg. per 100 ml. of plasma, then one must conclude that the frequently prescribed schedule of triple sulfonamide medication may in some patients give plasma concentrations 25 to 50 per cent higher than necessary. Second, although Benemid is capable of producing statistically significant elevations of free and total sulfonamide plasma concentrations, the biological significance of these elevations must be questioned, since the average enhancement with respect to the free and total components was only 2.2 and 4.6 mg. per 100 ml. of plasma, respectively. This latter statement is substantiated by the failure to observe any cumulative effect of Benemid on these levels.

## SUMMARY AND CONCLUSIONS

1. Triple sulfonamide preparations, with and without the use of Benemid, were administered to 12 individuals free of renal disease, as determined by routine measures.

2. Following the acute intravenous injection of a triple sulfonamide mixture, Benemid produced no change in free sulfonamide concentrations but a statistically significant elevation in total sulfonamide concentrations. The latter observation was due to an enhancement of the levels of the acetylated component.

3. Oral administration of a triple sulfonamide-penicillin preparation over a five-day period demonstrated the ability of Benemid to increase both the free and total sulfonamide levels as well as the resulting penicillemia.

4. There was no evidence of an accumulative phenomenon associated with the use of Benemid in conjunction with sulfonamide and/or penicillin.

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# NEWCASTLE DISEASE VIRUS IN MAN

## Results of Studies in Five Cases

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THIRTY-THREE cases of Newcastle disease were reported in man through 1950. These have been reviewed by Keeney and Hunter.[1] The virus was isolated in 7 cases. Human cases have been reported from Australia, Israel, Hungary, Maryland, Ohio, New Jersey, and Pennsylvania. Newcastle disease virus was recovered from a laboratory worker in Canada.[3] In 1951, an outbreak of conjunctivitis was investigated by the State of Minnesota Department of Health and the University of Minnesota Division of Veterinary Medicine.[4] The disease affected 40 of a total of 90 persons working on the eviscerating line of a poultry produce plant. Newcastle disease virus was isolated from material obtained from the eyes of 4 of 10 patients with acute symptoms.

Current methods for measurement of antibodies to Newcastle disease virus in human serum are unsatisfactory. The interpretation of results obtained by serologic tests alone does not justify the definitive diagnosis of infection with this virus in man.[2] Isolation of virus, without demonstrating a specific antibody rise in the serum, would also appear to be insufficient to establish the presence of infection. The problem is believed to be due in part to difficulties in the technical procedures incident to serologic tests in this disease. If these limitations are understood, descriptions of the circumstances in which Newcastle disease virus is associated with illness in human beings will probably add information of value.

The repository for strains of Newcastle disease virus at the University of Wisconsin includes 9 isolates obtained from human cases, 7 of which have not been reported* (Table I). The patients from which 3 of the isolates listed in Table I were recovered (W. A. C., E. L., and T. H.) were encountered at the University of Wisconsin. This report describes the clinical and laboratory observations made during these illnesses. The studies include what is probably the first instance of isolation of virus from urine and nasal discharges of man, the second time viremia has been demonstrated, and the first description of buccal mucosal lesions in human beings during an infection associated with Newcastle disease virus. Each patient had exposure in a laboratory.

### METHODS

Conjunctival washings from both eyes, discharges from the nose and mouth, and samples of urine of patients were centrifuged at 2,000 r.p.m. for fifteen minutes. An equal volume of an antibiotic mixture was added to the supernatant fluid and 0.1 ml. inoculated into the allantoic chamber of 10- to 11-day embryonating hen eggs. Each inoculum contained 250 units of penicillin and 250 micrograms of streptomycin. Either whole blood,

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*Maintained jointly by the Bureau of Animal Industry and the North Central Regional Technical Committee on Newcastle Disease at the University of Wisconsin.

TABLE I. LIST OF NEWCASTLE DISEASE VIRUS ISOLATES OBTAINED FROM HUMAN CASES

| ISOLATE* | ISOLATOR† |
|---|---|
| (H) Len—Mo.—1950 | H. C. McDougle, U. Missouri |
| (H) Davis—Ohio—1950 | W. L. Ingalls, Ohio State U. |
| (H) J. C. W.—Md.—1951 | J. C. Wagener, Camp Detrick |
| (H) M. S. W.—Md.—1951 | J. C. Wagener, Camp Detrick |
| (H) W. A. C.—Wis.—1951 | R. P. Hanson, U. Wisconsin |
| (H) E. L.—Wis.—1951 | R. P. Hanson, U. Wisconsin |
| (H) T. H.—Wis.—1952 | R. P. Hanson, U. Wisconsin |

*The isolate designation consists of the specific name followed by the abbreviation of the region or state of origin and the year of isolation.

†The worker making the isolation.

or the unwashed red cells, suspended in tryptose broth, also were used for inoculation, after addition of the antibiotic mixture. Six eggs were used for each isolation attempt. Prior experience has shown that at least this number of embryos should be used to detect small quantities of virus. The eggs were candled twice daily. Dead embryos were removed promptly after detection. Allantoic fluids were harvested and preserved at −20° C. for hemagglutination (HA), and other tests. The identity of the isolated virus was determined serologically by hemagglutination-inhibition and serum neutralization tests. In two instances the pathogenicity of the isolate for chickens was determined.

The patients' sera were inactivated at 56° C. for thirty minutes. Hemagglutination-inhibition (HI) and serum neutralization tests (SN), using allantoic fluid obtained from dead embryos, were performed as described by Brandly and associates.[5]

## CASE REPORTS

CASE 1.—W. A. C., a 25-year-old white man, reported on Oct. 9, 1951, complaining of irritation of the eyes. Three days previously, about 3:00 P.M., he had been exposed to a lyophilized preparation of chicken embryo infected with strain GB, Newcastle disease virus. The powder was blown into the air in his vicinity. He was without goggles or glasses. He remained well until the morning of October 8, about thirty-six hours after exposure, when itching of the eyes, which persisted, especially on the left, was noted. On awakening the next day, the left eye was "crusted," the conjunctivae were "inflamed," and mild malaise was present. The past history was otherwise noncontributory, and the physical examination was normal except for the eyes. There was slight edema of the lids, especially the left lower. The extraocular movements were performed well; the visual fields were normal, and the pupils reacted normally. The conjunctivae were diffusely injected, with slight follicular enlargement. Corneal lesions or keratitis were not demonstrated. There was no lymphadenopathy.

*Laboratory Examination:* Urinalysis and blood counts were within normal limits. The sedimentation rate (Wintrobe-Landsberg, corrected) was 11 mm. per hour. Microorganisms were not seen in gram-stained material from the conjunctival sac. Cultures of the material revealed *Staphylococcus aureus* on the right and *Staphylococcus albus* from the left. Inclusion bodies were not found by examination of conjunctival scrapings.

*Treatment:* Aureomycin ophthalmic ointment was administered locally four times the first day, and neomycin ophthalmic ointment substituted thereafter, four times daily for three days.

*Course:* On October 10, there was considerable photophobia. Watery lachrymal discharge and nasal discharge had increased. Preauricular nodes were enlarged and tender bilaterally. Periorbital edema was prominent, and the bulbar and palpebral conjunctivae had developed more edema and injection. The anterior pharyngeal pillars were injected. The buccal mucosa was superficially ulcerated and tender in an irregular area opposite the canine teeth bilaterally on the lateral and medial aspects. Fever of 100° F. was present. On October 11, malaise had diminished, but photophobia persisted. The vision was slightly blurred, and there was slight itching of the eyes. Examination was essentially as described on the previous day. The disease had subsided by October 16, except for slight palpebral

conjunctival injection and enlarged, tender preauricular lymph nodes. The chest roentgenogram of October 18 was negative. By October 23 there was slight blurring of distant vision, but no other symptoms. The only abnormality was enlargement and slight tenderness of the preauricular lymph nodes. One month after onset, the patient indicated by letter that some loss of accommodation was the only remaining symptom (Fig. 1).

*Results of Virus Isolation Studies:* Virus was isolated from washings from the right and left conjunctivae and from red blood cells, obtained on the third day after exposure. Virus was obtained from the saliva and nasal discharge, collected on the fourth day. Blood, saliva, urine, and eye washings, obtained ten days after exposure, did not contain virus (Table II).

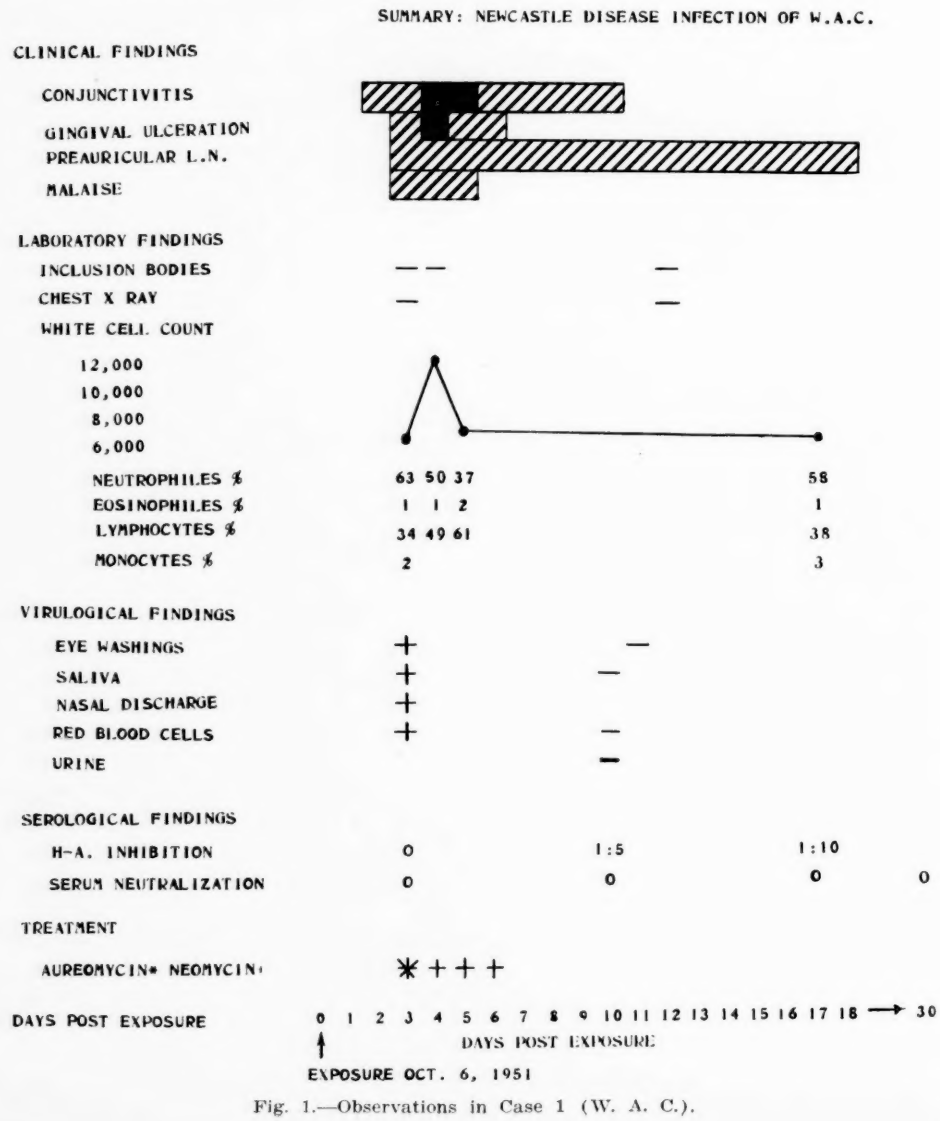


Fig. 1.—Observations in Case 1 (W. A. C.).

TABLE II. ISOLATION IN EMBRYONATING EGGS OF NEWCASTLE DISEASE VIRUS FROM THREE CASES OF HUMAN INFECTION

| SOURCE | DAYS POST EXPOSURE | NO. EGGS INOCULATED | NO. DEATHS IN 48 TO 96 HR. | NO. HA POSITIVE | INHIB. BY NDV ANTISERA | COMMENTS |
|---|---|---|---|---|---|---|
| W. A. C. washing, right eye | 3 | 6 | 6 | 6 | yes | ND Pos. |
| washing, left eye | 3 | 6 | 6 | 6 | yes | ND Pos. |
| blood cells | 3 | 6 | 1 | 0 | | * |
| saliva | 4 | 6 | 4 | 4 | yes | ND Pos.† |
| nasal discharge | 4 | 6 | 2 | 2 | yes | ND Pos. |
| whole blood | 10 | 6 | 0 | | | |
| saliva | 10 | 6 | 0 | | | ‡ |
| urine | 10 | 6 | 0 | | | ‡ |
| washings from eyes | 11 | 6 | 0 | | | |
| E. L. washing, left eye | 2 | 6 | 0 | | | |
| washing, right eye | 2 | 6 | 1 | 0 | | |
| urine | 2 | 6 | 0 | | | |
| saliva | 2 | 6 | 1 | 1 | yes | ND Pos. |
| blood | 2 | 6 | 1 | 0 | | |
| saliva | 3 | 6 | 3 | 1 | | ND Pos. |
| T. H. washing, left eye | 4 | 5 | 3 | 2 | yes | ND Pos. |
| washing, right eye | 4 | 5 | 5 | 2 | yes | ND Pos. |
| saliva | 4 | 4 | 4 | 1 | yes | ND Pos. |
| urine | 4 | 6 | 5 | 3 | yes | ND Pos. |

*Second egg passage fluids possessed hemagglutinins which were inhibited by NDV antisera.

†Induced fatal infection in chickens.

‡Noninfectious for chickens.

The isolate was highly pathogenic for 8-week-old chickens, as indicated by an $LD_{50}$ of $10^7$ per milliliter. Known Newcastle disease antiserum (Najarian strain) neutralized $10^{6.5}$ $LD_{50}$ of the isolate. A 1:1280 dilution of the serum inhibited 10 HA units of the isolate.

*Results of Serologic Studies:* Serum was obtained on the third, tenth, and eighteenth days after recognized exposure to the specific virus. There was a slight, but probably significant, rise in the hemagglutination-inhibition titer, from 0 on the third day to 1:5 and 1:10 on the tenth and eighteenth days, respectively. Serum neutralizing antibodies were not demonstrated.

CASE 2.—E. L., a 28-year-old white man, was seen on Nov. 30, 1951, complaining of a heavy sensation in the head and excessive watering of the eyes. These symptoms were not severe, and he would not have ordinarily reported them. However, two days previously he had inhaled an Aerosol spray, containing strain GB Newcastle disease virus, for forty-five minutes, while exposing chickens. The next day, November 29, he accidentally inoculated himself in the right wrist, to a depth of 2 to 3 millimeters, with a tissue culture of strain RO, Newcastle disease virus. The needle was attached to a syringe containing the virus. There was no history to suggest recent exposure to another infectious or irritating agent.

Physical examination demonstrated slight injection of the sclerae and bulbar conjunctivae. A small area of tenderness and injection of the gingival mucosa was noted above the right upper canine tooth. There was no lymph node enlargement. A small puncture wound, without surrounding edema or erythema, was seen on the flexor surface of the right wrist. Otherwise, the physical examination was within normal limits. At this time definite evidence of infection had not appeared. A complete blood count and urinalysis were normal.

On the next day, December 1, headache and mild sore throat appeared. The temperature was normal. The posterior pharyngeal mucosa appeared edematous and injected. The anterior superior cervical lymph nodes were slightly enlarged. The gingival mucosa above both upper canine teeth were injected. A throat culture was positive for beta hemolytic streptococci, group A, type 41. The clinical impression was acute pharyngitis, very mild, etiology unknown. By December 3, the headache had disappeared, but fatigability was present. The throat was less sore, but backache had developed. The eyes appeared normal. The anterior superior cervical lymph nodes were enlarged and slightly tender. The puncture wound was no longer visible. A chest roentgenogram was normal, and a throat culture was negative for beta hemolytic streptococci. No specific treatment had been prescribed. He recovered completely within the next two days.

*Results of Virus Isolation Studies* (Table II): Newcastle disease virus was isolated from saliva obtained on the second day after exposure (Nov. 30, 1951). Blood, conjunctival sac washings, and urine of that day failed to reveal the presence of virus. A known ND antiserum, in dilutions of 1:1280, inhibited 10 hemagglutinating units of the virus isolate.

*Results of Serologic Studies:* Sera obtained during the course of the illness and thirty-two days after onset did not reveal neutralizing or HI antibodies.

*In summary,* this patient was exposed to Newcastle disease virus by Aerosol spray and dermal inoculation. Mild acute pharyngitis occurred about seventy-two hours after inoculation. Newcastle disease virus was recovered from the saliva. On the same day beta hemolytic streptococci, group A, type 41, were obtained on culture of the throat. The mildness of the illness in this individual would seem to be noteworthy. The symptoms and signs would probably have been overlooked or disregarded by patient and physician in other circumstances. This case indicates that Newcastle disease virus can remain active in human beings in the absence of significant clinical manifestations. The relative significance of the beta hemolytic streptococcus and of Newcastle disease virus, isolated from pharynx and saliva, respectively, is in doubt.

CASE 3.—T. H., a 44-year-old white man, obtained medical attention because of itching and excessive lachrymation of both eyes. Four days previously he had washed several items of glassware which contained blood from about 700 turkeys, not known to have had Newcastle disease infection. Some of the glassware may have been contaminated by the blood of chickens which were known to have had the specific disease. The next day he noticed progressive itching and watering of the eyes. On examination, the bulbar and palpebral conjunctivae were moderately injected. Mild photophobia and excessive lachrymation were present. The sclerae were only slightly injected. Preauricular nodes were palpable, firm but not tender. The nasal, buccal, and pharyngeal mucosae were normal. The remainder of the physical examination was normal. The blood count and urinalysis were within normal limits.

*Course and Treatment:* Treatment consisted of a single installation of Butyn 2 per cent and Metaphen 1:3000 ophthalmic ointment, with boric acid eye washes twice daily for two days. Improvement was noted on the following day (three days after probable exposure to Newcastle disease virus). Seventeen days after presumed exposure, minimal lower lid edema and conjunctival injection remained. Symptoms had disappeared.

*Virus Isolation Studies:* Virus was isolated from saliva, conjunctival washings, and urine, obtained four days after presumed exposure. The isolate was identical by serologic test, to Newcastle disease virus (Table II). This strain was pathogenic for 8-week-old

chickens, as indicated by an $LD_{50}$ greater than $10^3$. Known ND antiserum (Najarian strain) neutralized $10^7$ $LD_{50}$ of the isolate. A 1:1280 dilution of antiserum inhibited 10 HA units.

CASE 4.—Another subject (W. P.) has been observed who developed conjunctivitis and pharyngitis after exposure to chickens with Newcastle disease. Newcastle disease virus was not isolated in this case.

W. P., a 26-year-old veterinarian, autopsied 4 chickens, 3 of which were obtained from a flock in which the disease was present. The serum of one contained HI antibodies. The patient used gloves during the autopsies. He did not recall being contaminated. The next day acute conjunctivitis on the left developed and became progressively more severe. After three days there was subjective and objective evidence of acute pharyngitis. Cultures of the pharynx and eyes were negative for common bacterial pathogens. The right conjunctiva and sclera became acutely inflamed during the six days after exposure. The patient had malaise, but remained afebrile. Conjunctivitis and pharyngitis disappeared in ten days. At this time the patient took a strenuous vacation trip. Conjunctivitis, with severe photophobia and pain, returned, and he was acutely ill for three days. The patient recovered gradually but completely during the week following.

Eye washings, nasal secretions, saliva, blood, and urine were obtained on four occasions during the stage of acute conjunctivitis. Newcastle disease virus was not recovered. Serum HI and SN antibodies were not demonstrated. On two occasions inclusion bodies of the lymphogranuloma type were present in a stained smear of conjunctival scrapings. This patient is described because of the similarity to other cases with conjunctivitis in association with Newcastle disease virus.

## DISCUSSION

Newcastle disease is one of the most important conditions affecting poultry throughout the world. It was recognized in chickens in the United States in 1941. The disease spread with damaging effectiveness, to become a major problem of the poultry industry in this country within a few years.

A few cases of Newcastle disease have been reported in man during the past four to five years. However, its potentialities did not become fully realized until recently. The evidence obtained suggests that this virus is capable of causing a systemic infection in human beings. Demonstration of virus in saliva, nasal discharges, and conjunctival sac washings indicates possibilities for human being to human being transfer.

Proof of human infection with Newcastle disease virus is difficult to establish. On several occasions, the specific virus has been isolated from human beings in the absence of a rise in serum hemagglutination-inhibition or neutralizing antibodies, as measured by the methods available. Other investigators have been unable to demonstrate an immunologic response in individuals from whom ND virus had been isolated.[4, 7] The discrepancy has been attributed in part to difficulties in technical procedures. Immunization records of W. A. C. indicated that this subject had responded poorly to several immunogens in the past.[8] In the cases reported here, the history of exposure and somewhat uniform clinical course subsequently, with isolation of the specific virus during the illness, provides suggestive evidence for an etiologic relationship. Other reports support this view. A strain of Newcastle disease virus has been isolated from human lung tissue.[9]

Conjunctivitis was the principal feature in four of the patients reported here, and in all but one of those previously described. W. A. C. and E. L. had ulcerations of the buccal mucosa. Evidence was lacking to suggest involvement of the lungs, gastrointestinal tract, or central nervous system. Isolation of virus from blood and urine indicates systemic dissemination which could afford opportunity for invasion of any tissue.

Newcastle disease may have potentialities for widespread dissemination in man, with resultant important significance to public health. Inapparent infection is a possibility (suggested by Case 2). A comment, made in 1946, by Dr. F. M. Burnet is appropriate, "From the human standpoint, the ancestry of Newcastle disease virus is of less importance than the possibility that it, or some similar virus, might on occasion find opportunity for human passage and by appearance and selective survival of appropriate mutants give rise to a new antigenic type of influenza."[6]

During the week of Feb. 26, 1952, a brief survey was made of the personnel of a near-by commercial firm, which is engaged in making vaccine for the prevention of Newcastle disease in chickens. In the establishment, 8 individuals have frequent opportunities for contact with active virus. One of them joined the other 7 about two weeks before. He was ill at the time of survey. This individual manifested clinical features identical to those described. Newcastle disease virus was isolated from eye washings. Saliva, urine and blood were negative. Within the past eight months the other 7 persons had had a similar illness. All of them consider infection with this specific virus to be expected in the course of their occupation. Proof of this is lacking at present, but the circumstances are suggestive and would seem to justify further study.

## SUMMARY

1. The history and clinical course of illness of 5 patients who were exposed accidentally to Newcastle disease virus is presented.

2. The principal clinical manifestations were malaise, conjunctivitis, ulceration of the buccal mucosa, and preauricular lymphadenopathy. The onset followed exposure within eighteen to twenty-four hours.

3. Newcastle disease virus was isolated from conjunctival sac washings, nasal discharges, saliva, and blood cells of Case 1, from saliva only in Case 2, from conjunctival sac washings, saliva, and urine in Case 3, and from eye washings of a patient whose contact occurred while preparing vaccine to Newcastle disease.

4. As far as known, Newcastle disease virus was isolated for the first time from urine of a human patient.

5. The virus was isolated from one patient whose illness was almost inapparent.

6. It is suggested that Newcastle disease may have greater significance for man than is generally recognized.

The authors gratefully acknowledge the technical assistance of Dorothy Dow.

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# OBSERVATIONS ON LAENNEC'S CIRRHOSIS: THE EFFECTS OF CORTISONE ACETATE DURING LOW-SODIUM REGIMEN

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## INTRODUCTION

THE present methods of treatment of cirrhosis of the liver are satisfactory in that they provide a metabolic mixture of foods which allow the liver cells to regenerate. Moreover, they correct disturbed nutrition and endocrinological abnormalities. During the past few years the management of water and electrolyte disturbances has also improved.[1, 2] On the other hand, there is no satisfactory method of getting rid of the connective tissue overgrowth which is responsible for cirrhosis.

Adrenal hormones are known to block the deposition of abnormal mesenchymal deposits, and we therefore thought it worth while to see if long-term treatment with cortisone would be of value in resolving abnormal connective and fibrous tissue in the livers of cirrhotic patients. In order to do this a series of detailed, short-term metabolic studies had to be carried out to observe the effect of cortisone upon patients with chronic liver disease. Three patients with cirrhosis were treated with cortisone for eighteen days while subsisting on a diet low in sodium, and our observations on them are reported in this communication.

## METHODS

1. *Patients.*—Space does not permit detailed case histories of the three patients studied. They were studied in the metabolic unit of the Research and Educational Hospitals, and gave histories of chronic alcoholism and dietary negligence. On admission two of the men presented with hepatomegaly, splenomegaly, ascites, and jaundice (R. D., J. N.). In the third man (W. McD.), the liver and spleen were firm and enlarged. All had numerous vascular spider nevi in the head, neck, upper trunk, and arms, dilated abdominal wall subcutaneous veins, and internal hemorrhoids. The dry weight of the two subjects with ascites (R. D., J. N.) was markedly under average for their height and age. The third man (W. McD.) was obese. None of the patients had bled from esophageal varices or peptic ulcers.

Clinical and detailed laboratory and radiologic examination showed no evidence of illness concurrent to hepatic cirrhosis. Routine blood chemistries and liver function tests revealed only abnormalities consistent with cirrhosis. Prior to the studies the patients were tested by salt loading, and all showed a definite increase of body weight with retention of sodium and water.

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2. *Laboratory Techniques.*—In the ward the patients consumed a nutritionally well-balanced diet with fixed caloric, protein, sodium, potassium, calcium, phosphorus, and ascorbic acid intake as indicated in Figs. 1, 2, and 3. Neither vitamin capsules nor other nutritional supplements were used in therapy. Fluid intake was also fixed at 2.5 liters per day. The environment was kept constant in rooms with a temperature of 70° F. and 40 per cent relative humidity. Metabolic balance studies were made by chemical analyses of food, feces, and urine. Nitrogen, soduim, potassium, phosphorus, calcium, uric acid, and creatinine were determined by standard procedures recorded elsewhere.[3] Ascorbic acid was determined by the method of Roe and Kuether.[4]

Urinalysis with microscopic examination was done daily. Standard hematologic studies were done three times a week on venous blood. The eosinophils were counted using a modification of Randolph's method.[5] Blood glucose, nonprotein nitrogen, carbon dioxide combining power, cholesterol, and cholesterol esters were determined by standard techniques.[6] The total proteins of serum were determined by the micro-Kjeldahl method.[7] Serum albumin was determined by a modification of the Howe method.[8] The liver function tests employed have been listed elsewhere.[3] Urinary 17-ketosteroids were measured by the method of Robbie and Gibson.[9]

3. *Therapy.*—After a control period in which metabolic data were completed, each subject was given 100 mg. of cortisone acetate by the intramuscular route daily for eighteen days. This eighteen-day period was followed by a second eighteen-day study period to observe the effect of withdrawal from treatment.

## RESULTS

Since each of the patients responded in an individual manner to therapy with cortisone, the results of therapy will be listed separately. Metabolic balances of nitrogen and electrolytes, 17-ketosteroid excretion, urinary output, body weight, eosinophil counts, and urinary uric acid-creatinine ratios for each individual are shown in Figs. 1, 2, and 3.

Case 1.—Patient W. McD. Data on this patient are shown in Fig. 1. During the control period the patient was in nitrogen balance for eighteen days, and cortisone acetate, 100 mg. by the intramuscular route, was started on the nineteenth day and continued through the thirty-sixth day. There was no change in the weight of the patient during the latter half of the therapy period; however, he excreted more urine than during the control period. The liver size and firmness increased remarkably after the seventh day of therapy. No changes in body temperature or pulse rate occurred. Depression and paranoid trends appeared late in the treatment period.

There was no significant change in hematocrit, hemoglobin, or erythrocyte count. The leukocyte count remained in the low normal range, but the differential count changed from 70 per cent segmented cells and 28 per cent lymphocytes to 48 per cent segmented cells and 49 per cent lymphocytes. The total eosinophil count did not change in any characteristic manner. Concerning the liver function tests, there was a fall in gamma globulin from 1.10 to 0.89 units during therapy, but no effect on the other tests of this group occurred. The uric acid-creatinine ratio rose but slightly. A slight rise in urinary 17-ketoteroid excretion appeared from 20 mg. per day to 25 mg. per day (Fig. 1). Serum albumin rose from 3.5 to 3.8 Gm. per cent, and the total protein rose from 5.0 to 6.0 Gm. per cent early in therapy. An initial rise of the NPN from 38 to 51 mg. per 100 ml., and $CO_2$ combining power of the blood appeared from 50 to 58 volumes per cent. There were no striking changes in serum sodium and calcium levels, but a sustained rise in serum potassium from 3.5 to 4.7 meq. per liter and a continuous fall in serum phosphorus from 4.1 to 2.8 mg. per 100 ml. occurred.

Metabolic balance data during therapy are shown in Fig. 1. The patient went into a slight negative nitrogen balance of 15 Gm. for the eighteen days. There was increased sodium excretion toward the end of therapy. The potassium balance did not change remarkably from

the last part of the control period. Calcium balance became increasingly positive. The phosphorus balance was less negative than it had been during the control period. There was no change in ascorbic acid excretion.

In the eighteen days following the end of cortisone therapy there was a lower urinary output except for the first three-day period. The liver returned to its normal size one week after cessation of therapy. There was no change in blood pressure or vital signs. The patient's mental aberrations which had appeared during therapy gradually disappeared.

The hematocrit fluctuated from 37 to 42, and dropped to 39 per cent. The total leukocytes remained in low normal range. The percentage of segmented cells rose from 48 to 57 fifteen days after stopping cortisone, and reciprocally the lymphocytes dropped from 52 per cent on the third day to 41 per cent on the fifteenth day. Urinalysis findings did not

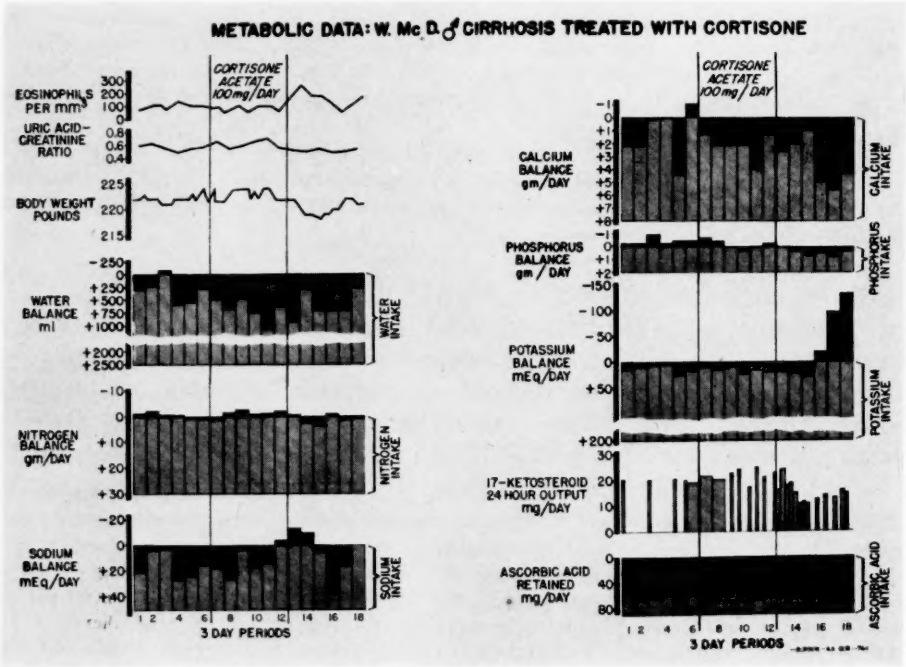


Fig. 1.—The effects of treatment with cortisone on the eosinophils, uric acid-creatinine ratio, body weight, urinary 17-ketosteroid output, and metabolic balances of water, nitrogen, sodium, calcium, phosphorus, potassium, and ascorbic acid in patient W. McD. aged 45 years, ill with cirrhosis of the liver. The solid black areas represent positive balance. The diagonally striped areas represent total urinary and fecal output. The cross-hatched areas represent negative balance.

change. Among the ten liver function tests there was only a rebound rise in the gamma globulin values from 0.9 to 1.3 units. There was a decrease in total cholesterol from 161 to 120 mg. per 100 ml. and cholesterol ester from 125 to 36 mg. per 100 ml., and a rise in serum potassium from 4.5 to 4.8 meq. per liter, otherwise no notable changes in the blood and serum chemistry levels were seen.

During this time the uric acid-creatinine level declined slightly, but there was a marked increase in the total eosinophil count from 66 to 264 per cubic millimeter, and a marked decrease in urinary 17-ketosteroid output.

Nitrogen balance became positive. The sodium balance was positive, but then became negative; the potassium balance did not change during the first half of the eighteen-day period; it then became strongly negative. These alterations in electrolyte balance occurred

without any change in serum values for sodium and potassium. The calcium and phosphorus outputs decreased progressively during the final nine days. No change was noted in ascorbic acid excretion.

*Comment.*—(Fig. 1.) Aside from changes in the gamma globulin and serum albumin values there is no evidence of benefit of the patient's cirrhosis during cortisone therapy. On the contrary, the patient was in negative nitrogen balance during therapy and his liver increased in size and became very firm. Various hematologic and metabolic features of the study are merely characteristic of what may possibly happen with cortisone therapy.[10]

CASE 2.—Patient R. D., aged 32 years. After an eighteen-day period during which the patient was in positive nitrogen and calcium balance, therapy was started with cortisone acetate. Although he had been given a salt-loading test during the fourth, fifth, and sixth days of the control period, his weight curve and water, sodium, and potassium output had stabilized before the end of the control period. During this time the ascites had disappeared and his nutrition was improving rapidly. During salt loading there was a drop in the patient's serum cholinesterase activity from 0.60 to 0.42 unit, and the ascorbic acid excretion increased temporarily. There was no significant changes incidental to salt loading in any of the liver function tests (except for a fall from 0.56 to 0.47 unit in the serum cholinesterase activity) nor in any of the blood or serum chemical values.

Fig. 2 reveals that the increase in body weight was less rapid during therapy, and in the latter half of the period there was an increased output of urine. The patient's liver became larger and was more firm during the latter part of therapy. He became ravenously hungry and demanded more food. He threatened to leave the hospital. This was prevented by increasing his diet to 3,725 calories, 150 Gm. of protein, 125 Gm. of fat, and 500 Gm. of carbohydrate (see Fig. 2, twelfth three-day period). On the last day of treatment the patient had a moderately severe acneform eruption on his face and on the upper chest, both anteriorly and posteriorly. His libido had increased and gynecomastia appeared. His beard began growing more rapidly and body hair began to increase. The nutritional status continued to improve.

On the second and fourth days of treatment the patient's temperature rose to 101° F. and 100° F., respectively; these febrile episodes were accompanied by chills. Nose, throat, blood, and urine cultures were negative for pathogenic organisms, but the patient had tenderness over his maxillary sinuses. He had a leukocytic response at this time with from 6,000 to 13,000 over a period of five days. He received no added medication, and the fever and chills subsided spontaneously, but the leukocyte count persisted at a range between 12,000 and 15,000 per cubic millimeter until discontinuation of therapy. No changes were present in the daily urinalysis or blood pressure measurements. Among the liver function tests there was a slight drop in cholinesterase from 0.53 to 0.43 which was sustained, and a transitory drop in gamma globulin from 1.03 to 0.64, which returned to 1.11 units. The serum potassium rose from 4.0 to 4.3 meq. per liter, otherwise there were no changes in blood and serum chemical values.

There was a gradual increase in the urinary uric acid-creatinine level as seen in Fig. 2, but no important difference in the total eosinophil count of the 17-ketosteroid output.

The nitrogen balance became negative during the latter half of the period; the sodium and potassium output exceeded the intake, and the calcium balance became negative. No change in the phosphorus output occurred. The ascorbic acid excretion was noticeably increased. These changes are seen in Fig. 2. After therapy the patient's appetite was still increased, but his increased diet was satisfying him. His liver appeared to revert to its former size and consistency about a week or ten days after cessation of therapy. There was improvement of the acne but no change in the beard, body hair, or slight gynecomastia. The last finding persisted for some weeks and gradually regressed. The leukocyte count returned to 5,000 and the segmented cells decreased from 70 to 50; the lymphocytes increased

from 25 to 46. There were no remarkable changes in the urinalysis findings. There were no definite changes in the liver function tests or blood and serum chemistries. One exception to the latter was the NPN which rose from 25 to 37 mg. per cent temporarily after the dietary protein was increased on the fifteenth day of therapy.

There was a rebound rise of eosinophils from 149 to 484 per cubic millimeter, and the urinary uric acid-creatinine ratio fell early in the post-treatment period (Fig. 2). After the fifteenth day of cortisone therapy the metabolic balance data and water output and body weight are not suitable for exact interpretation due to the introduction of a new diet in the middle of the study. This is also true for the ascorbic acid excretion studies.

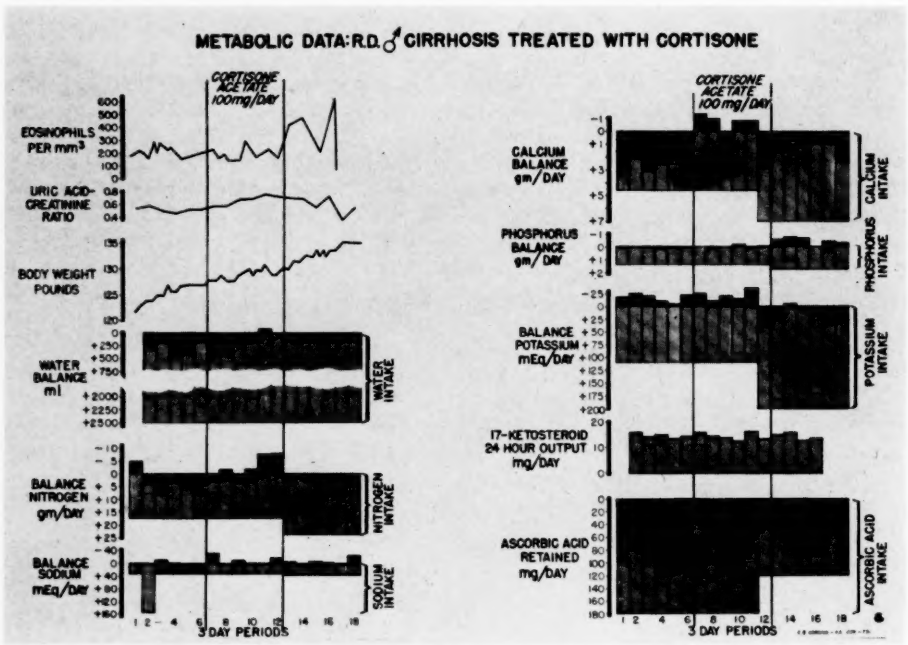


Fig. 2.—The effects of treatment with cortisone on the eosinophils, uric acid-creatinine ratio, body weight, urinary 17-ketosteroid output, and metabolic balances of water, nitrogen, sodium, calcium, phosphorus, potassium, and ascorbic acid in patient R. D., aged 32 years, ill with cirrhosis of the liver. The solid black areas represent positive balance. The diagonally striped areas represent total urinary and fecal output. The cross-hatched areas represent negative balance.

*Comment.*—(Fig. 2.) The patient was a young man who was improving rapidly in his nutritional and endocrinologic rehabilitation at the time treatment was started. During the first few days of treatment the patient apparently developed an acute sinusitis with fever and chills, and pain in his face; this subsided without treatment and, although he had a leukocyte response, no bacteria were recovered from cultures. During the latter part of treatment he experienced hunger pains during the night and his diet had to be increased lest he leave the hospital under protest. An increase in the liver's size and firmness, and an acneform rash with characteristic distribution appeared late in treatment and regressed in the post-treatment period. Return of libido, growth of beard and body hair, and development of gynecomastia appeared during cortisone therapy, and these changes persisted after cessation of cortisone

administration. This was probably related to his improved nutritional state and is an instance of refeeding gynecomastia in cirrhosis of the liver which one of us has reported elsewhere.[11]

The serum cholinesterase activity decreased during and after therapy, and the gamma globulin value dropped transitorily and initially during cortisone administration; other liver function tests did not change. (Body weight, serum albumin values, and the forty-five minute bromsulfalein retention improved, apparently regardless of therapy.) The serum potassium rose and remained stable in the latter part of therapy and after withdrawal of cortisone.

Vigorous reversal from positive to negative nitrogen and calcium balances occurred when cortisone was started. This is not unusual during cortisone therapy.[10] The sodium balance became negative during treatment, but the dietary intake of sodium was carefully restricted to 0.73 Gm. a day before the diet was changed, and to 0.9 Gm. a day after the change in diet. The post-treatment balance studies are probably not suitable for interpretation because the diet was changed.

Ascorbic acid excretion increased markedly during cortisone therapy. A sodium loading test ending fifteen days before therapy had indicated the patient was able to tolerate rather large amounts of sodium, after temporary sodium retention (Fig. 2).

CASE 3.—Patient J. N., aged 58 years. This patient could not subsist on the same dietary levels as the other two men. When this had been attempted he was found to retain so much water and sodium that he required peritoneocentesis. This was done eight days before the start of therapy. He was therefore fed on a fixed diet very low in sodium (585 mg. per day). The patient was in slight negative nitrogen balance, marked sodium retention, negative potassium and phosphorus balance, and positive calcium balance before therapy. During therapy, urine output increased during the latter part of the period, regardless of body weight increase and a fixed low maximum sodium output. Due to the gradual accumulation of ascitic fluid, the liver mass could be detected only by ballottement, and semiquantitative estimation of liver size was not possible. No changes in blood pressure, pulse rate, or body temperature were apparent. Increased appetite appeared during the latter part of the period along with emotional depression. No changes in hematological or urinary examination occurred. As shown in Fig. 3, the urinary uric acid-creatinine ratio increased during the middle portion of the period only; at the same time the urinary 17-ketosteroid excretion showed an increase which was sustained. There were no definite trends present in the total eosinophil counts.

Serum cholesterol values rose from 193 to 236 mg. per 100 ml. and thirty-minute bilirubin values decreased from 3.5 to 2.9 mg. per 100 ml. The NPN rose from 34 to 41 mg. per 100 ml., and carbon dioxide combining power of the blood rose from 46 to 57 during therapy.

The nitrogen balance became equal and the potassium and phosphorus balances became positive. The potassium balance became negative in the final three days. No changes appeared in the sodium and calcium excretion. Ascorbic acid excretion rose sharply and the rise was sustained (Fig. 3).

On the fifth day following the withdrawal of cortisone, the patient's temperature rose daily for five days, the daily peaks ranging from 100° to 102° F. Repeated blood, nose, sputum and throat, and urine cultures were negative. The patient had no diarrhea. He accepted his diet regardless of the fever. No additional therapy was given, and the fever subsided spontaneously. The patient had no pain. No rise in leukocytes or segmented cells occurred during the febrile period. Urinary output was suppressed and the body weight rose rapidly (Fig. 3). The patient became somewhat lethargic but experienced no marked

psychological reaction to the withdrawal of therapy or the intercurrent febrile episode. There was no change in the microscopic findings of the urine. Zinc sulfate turbidity changed from 10.0 to 9.5 units, gamma globulin values rose from 2.9 to 5.0 mg. per 100 ml.

Urinary uric acid-creatinine ratios rose and total eosinophils decreased following the onset of the febrile episode, but had no changes directly related to withdrawal of the drug. Urinary 17-ketosteroid excretion did decrease following the end of cortisone therapy (Fig. 3).

Following the onset of the febrile episode a negative nitrogen balance and slight potassium retention occurred. Otherwise no prominent changes occurred in the metabolic balance; the positive calcium and phosphorus balances persisted, and the sodium excretion remained low (Fig. 3). Ascorbic acid output decreased immediately approaching the pre-treatment level, with a small secondary rise incidental to the febrile episode (Fig. 3).

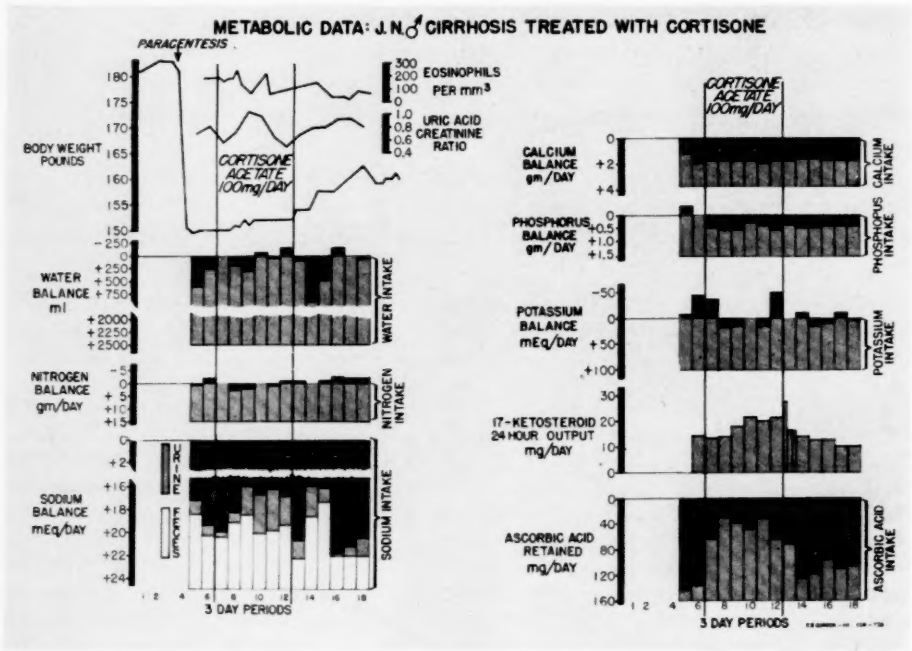


Fig. 3.—The effects of treatment with cortisone on the eosinophils, uric acid-creatinine ratio, body weight, urinary 17-ketosteroid output, and metabolic balances of water, nitrogen, sodium, calcium, phosphorus, potassium and ascorbic acid in patient J. N. aged 58 years, ill with cirrhosis of the liver. The solid black areas represent positive balance. The diagonally striped areas represent total urinary and fecal output. The cross-hatched areas represent negative balance.

*Comment.*—In evaluation of the data, two events make interpretation difficult: treatment was begun eight days following paracentesis, and the patient had a fever of unexplained origin from the fifth to the ninth day following withdrawal of cortisone. Regardless of this, the patient's urine output increased during the latter part of the treatment period and his appetite increased during therapy. He demonstrated no obvious endocrine changes. A rise of serum cholesterol and a decrease in serum total bilirubin values occurred during therapy. A positive nitrogen balance became negative with cessation of cortisone, and phosphorus balances were sustained during and after treatment. Ascorbic acid excretion increased markedly during therapy only.

*Correlation of Cases.*—All three patients increased their urinary output during the last nine days of treatment with cortisone. In two there was a simultaneous increase in urinary sodium output. The liver size and consistency could be followed satisfactorily in two of the men and was observed to increase in size during treatment. This has been reported previously.[12]

Various hematologic features were observed and noted. Two had a rebound increase of total eosinophils after therapy. Gamma globulin decreased slightly during therapy. Two men had an increase of serum albumin starting during therapy and sustained after its withdrawal. One man had a decrease in serum total bilirubin during therapy. All patients had an increase of appetite. Two became depressed; one of these developed paranoid ideas. One patient developed acne and a noticeable increase in beard and body hair.

## DISCUSSION

Cortisone does not usually produce a sodium and water diuresis, even when patients are living on low-sodium diets. In two of the patients the sodium output increased during therapy with cortisone, and in all three patients urinary output increased from the tenth day of treatment until it was stopped eight days later. No measurements were made of insensible water loss or of water loss by perspiration or respiration, and therefore it is impossible to give exact data on water balance. However, water and dietary water intake was fixed while the patients were living in a fixed environment at 70° C. There was no obvious perspiration or change in respiration rate (save in patient R. D. during a mild febrile reaction) nor was there loss of water through vomitus or diarrhea. Thus the diuresis could be the result of a considerable negative water balance with loss of extracirculatory water, or it could be caused by a shift in water excretion, the result of gross depression of insensible water loss. We doubt if the latter explanation is correct, and suggest that cortisone therapy was responsible for diuresis and loss of edema fluid. We can only speculate on the mechanism of this favorable effect of cortisone. Perhaps its use inhibited or depressed production of either the antidiuretic hormone of the posterior pituitary[13] or ACTH.[14] If the former were true one would have expected to find a decrease in urinary specific gravity at the time of diuresis. This did not occur. Inhibition of adrenocortical activity by ACTH depression, which is common with cortisone therapy, may have reduced mineralo-cortical activity as we have suggested elsewhere.[15] This would produce diuresis with loss of sodium which occurred in patient R. D. and at the end of the therapy period in W. McD.

The other favorable effect of cortisone therapy was the observation of a decrease in gamma globulin levels in two patients. If one accepts Popper's contention[16] that high gamma globulin levels reflect abnormal mesenchymal activity in the liver—and we do—then this indicates that cortisone is effective in changing a central mechanism which is responsible for cirrhosis.

Unfortunately these good effects of cortisone therapy were offset by its unfavorable effect on reparative phenomena (negative nitrogen balance, decreased serum cholinesterase) and by its unfortunate side effects (mental changes, fever, and hepatic enlargement). These will be discussed below, together with other observations.

With cortisone treatment, two of our three patients had a definite increase of liver size, and the increase was less definite but likely in the third patient who had ascitic fluid accumulation during therapy. There was reversion to approximately former size of the liver within ten days after cessation of treatment. Two patients (patients W. McD. and R. D.) had an immediate fall in gamma globulin, and this was sustained in one (patient R. D.). One patient had a fall of serum bilirubin from 1.17 to 0.94 mg. during treatment with a rebound to 1.95 mg. per cent afterward. The serum cholesterol had no characteristic change in level and/or trend during treatment, but in W. McD. in the second week following cessation of treatment the cholesterol value fell with a simultaneous rise in free cholesterol. Serum albumin increased and globulin decreased in W. McD. during cortisone therapy, and the rise was sustained; the other two subjects revealed no change in level and/or trend of serum albumin or globulin. Serum cholinesterase was depressed in R. D. during a cortisone therapy from 0.53 to 0.43, rising again to 0.49, fifteen days after withdrawal from cortisone treatment.

Gyorgy and Bleumle[17] report an increase in liver size, increase in urinary urobilinogen (Watson two-hour test); and decrease in thymol turbidity, Kunkel gamma globulin turbidity, bromsulfalein retention, and 17-ketosteroid excretion in patients with liver disease who received cortisone. Butt and his associates[18] report two cases of concurrent liver disease and rheumatoid arthritis, one with metabolic studies. They cite from various authors a fall in zinc sulfate turbidity and rise in serum cholesterol in patients with different diseases, and further instances of a fall in serum bilirubin and Kunkel gamma globulin turbidity in patients with liver disease receiving ACTH. In their own two cases one had a temporary fall of serum bilirubin, and both patients had further depression of an initially low 17-ketosteroid excretion; neither patient showed any change in his lipid metabolism.

During low-sodium, high-protein intake the effects of cortisone on nitrogen and electrolyte excretion are found to vary somewhat from patient to patient. R. D., a young man who had improved rapidly from a severely ill state, was found to have a typical or exaggerated response to therapy with negative balances in nitrogen, sodium, potassium, and calcium, all of striking magnitude. Although W. McD. had high negative nitrogen and potassium balances and a negative sodium balance during the latter part of therapy, his most striking responses were after therapy, with early rebound decrease in sodium excretion, continual decrease in phosphorus output, and later negative balance for potassium and positive balance for calcium. J. N. had no change in output of electrolytes that could be attributed to cortisone, although during its use he had definite increases of the 17-ketosteroid and urinary uric acid-creatinine ratio.

There is no correspondence of the actual nitrogen balance and theoretical balances[19] derived from potassium or calcium and phosphorus in any of these cases. This effect of adrenocortical hormones was first demonstrated by Forsham and associates.[20]

Kark and Johnson[21] studying soldiers under stress (a three-month march through the Arctic) found a marked retention of urinary ascorbic acid at levels

well below the saturation point of intake. Beck and his colleagues[22, 23] find marked increase of ascorbic acid excretion in patients receiving ACTH at both high and low levels of ascorbic acid intake, although the incidence of this phenomenon was less in the latter group. They find only one instance among nine patients of an immediate definite increase of ascorbic acid excretion, and decrease in this was not found in any of the patients upon withdrawal of therapy. Cortisone apparently had no effect. However, two of our subjects, R. D. and J. N., show a rise in ascorbic acid excretion during therapy with a decrease of output following the end of therapy.[24] The blood and serum chemistries revealed no other striking departure from the reports of others.

All three patients had normal 17-ketosteroid excretion levels before use of cortisone, which was contrary to the findings of Gyorgy and associates[17] and Butt and associates,[18] who found a decrease in 17-ketosteroids. Patients W. McD. and J. N. had a slight increase during therapy and decrease after withdrawal.

In healthy subjects the use of cortisone usually produces a rapid and sharp rise of 17-ketosteroid excretion.[10] This can be interpreted as evidence that cortisone is transformed in the body to 17-ketosteroids. In our patients with liver disease the 17-ketosteroids did not change remarkably during therapy. This suggests that normally the liver is concerned in the metabolic degradation of adrenal hormones as with other hormones.[25]

## SUMMARY

Three patients ill with Laennec's cirrhosis due to chronic alcoholism and poor nutrition, living on a fixed low-sodium, high-protein regimen, were treated with 100 mg. of cortisone acetate given intramuscularly each day for eighteen days. Observations of clinical signs, hematologic tests, urinalyses, daily urine volumes, metabolic balances of sodium, potassium, calcium, and phosphorus, serum and blood chemistry, liver function tests, total eosinophil counts, 17-ketosteroid excretions, urinary uric acid-creatinine values, and ascorbic acid content of the urine and feces are reported.

Urinary output increased in all patients during the last nine days of therapy and in two there was noted a simultaneous increase in urinary sodium output. In two patients, an increased excretion of ascorbic acid was found during cortisone therapy. The 17-ketosteroid excretion did not change during therapy, which suggests that the liver is concerned in the degradation of adrenal hormones. Aside from these changes, apparently cortisone therapy did not produce metabolic effects unique to cirrhosis.

With therapy there were minor changes in the various liver functions tested, and in each patient the liver enlarged rapidly and became very firm.

Cortisone therapy did not appear to affect the clinical or metabolic status of the patients permanently. Within two weeks of stopping therapy all the observed effects of the hormone had worn off.

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# LABORATORY METHODS

## A METHOD FOR THE SIMULTANEOUS QUANTITATIVE DETERMINATION OF CHOLIC ACID AND DESOXYCHOLIC ACID

### I. Application to Bile

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CHOLIC acid and desoxycholic acid, the predominating human bile acids, differ widely in their toxicity,[1-3] hemolytic properties,[4] effects on cholesterol metabolism,[5] and alterations in pathologic conditions.[6]

Bile acid methods in use at the present time either do not distinguish between cholic acid and desoxycholic acid or measure only cholic acid[7-13] Some methods are too time consuming or too insensitive to make them readily adaptable to the estimation of small quantities of the bile acids. The purpose of this paper is to present a method for the simultaneous quantitative determination of desoxycholic acid and cholic acid and to illustrate its application to bile.

#### MATERIALS AND EQUIPMENT

The cholic acid and desoxycholic acid used in these experiments were obtained from Hoffman and LaRoche. The former melted at 196° C., the latter at 176° C. Sabotka reports melting points of 196 to 197° C.[14] and 176° C.,[15] respectively, for these products. The sulfuric acid was of analytical reagent grade and was made up to 65 per cent by volume. The absorption spectra were read on the Beckman DU Quartz Spectrophotometer with the ultraviolet attachment. Matched silica cells were used.

Several solvent combinations and techniques were investigated. Chromatographic adsorption on alumina oxide,[16] 6.7N hydrochloric acid extract of an ether solution of bile acids,[17] and several modifications of Harwood's technique[18] using zinc hydroxide were tried but found unsatisfactory. Numerous combinations of ethyl ether, ethyl alcohol, acetone, chloroform, and petroleum ether were tested. The chief difficulties encountered were the introduction of extraneous interfering substances, the extreme heat lability of the bile acids, and obtaining maximum extraction of desoxycholic acid and cholic acid simultaneously. The fluid found best adapted for the simultaneous quantitative extraction of desoxycholic acid and cholic acid was a mixture of absolute alcohol, acetone, and ether (1:1:1).

It was necessary to redistill all solvents in all-glass apparatus to reduce extraneous interfering substances to a minimum. The filter paper used was ether extracted.

#### EXPERIMENTAL PROCEDURE

Qualitative studies by Bandow[19] showed that a maximum ultraviolet absorption of cholic acid and desoxycholic acid was obtained in 65 per cent sulfuric acid solution. A 65

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per cent sulfuric acid solution produced curves with absorption maximum at 3,100 Å and 3,800 Å. At 3,100 Å cholic acid had a greater absorption than desoxycholic acid, while desoxycholic acid had the greater absorption at 3,800 Å.

Ten milligrams of cholic acid or desoxycholic acid were quantitatively transferred to a 100 ml. volumetric flash and made up to volume with absolute alcohol. One milliliter of the alcohol solution (containing 100 μg) was pipetted into a 50 ml. beaker. The solution was evaporated to dryness on a hot water bath and allowed to cool to room temperature. Five milliliters of 65 per cent sulfuric acid were added, and the optical density was read. All readings were compared with a 65 per cent sulfuric acid blank.

Ultraviolet absorption curves were established by measuring the optical density of 100 μg of cholic acid and desoxycholic acid at wave lengths ranging from 2,300 Å to 4,400 Å (Fig. 1). The curves obtained agree with Bandow's results. The absorption wave lengths considered most suitable for these experiments were 3,180 Å for cholic acid and 3,500 Å

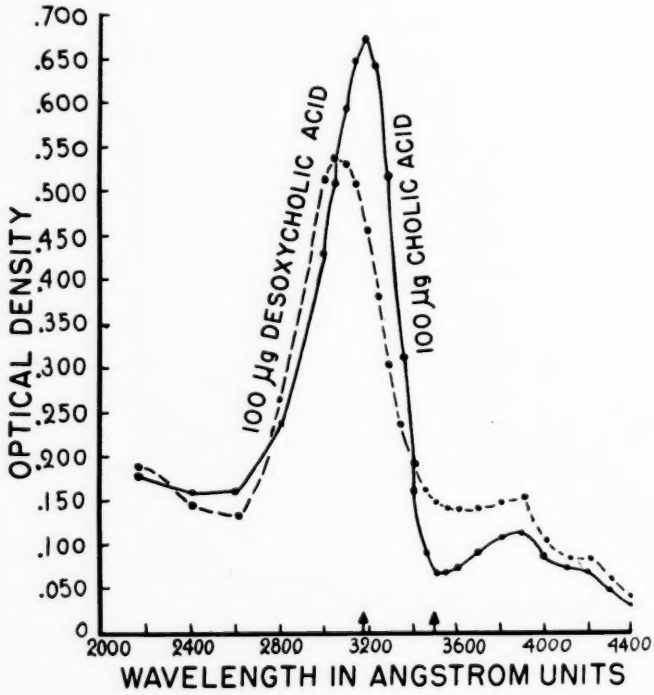


Fig. 1.—The ultraviolet absorption curves of cholic acid and desoxycholic acid in 65 per cent sulfuric acid.

for desoxycholic acid, since the optimum absorption difference occurs at these points, with cholic acid having the greater relative absorption at 3,180 Å and desoxycholic acid having the greater relative absorption at 3,500 Å. These respective wave lengths when changed ± 5 Å had negligible effect upon the intensity of absorption, so that a possible error in wave length setting would have minimal effect.

Observations upon the effect of time from the addition of the sulfuric acid upon the optical density of the solutions were noted (Fig. 2). The period of maximum stability proved to be forty to fifty minutes.

Suitability of this ultraviolet absorption technique for a quantitative assay basis was confirmed by demonstrating that the Beer-Lambert law was obeyed when concentrations of 50, 100, and 200 micrograms of cholic acid and of desoxycholic acid were measured and plotted against the average optical density (Fig. 3).

From the known optimum differences in absorption of cholic and desoxycholic acid at 3,180 Å and 3,500 Å, it is possible to calculate the concentrations of each in a mixed sample whose optical density has been determined at these wave lengths.

The following are essentially applications of a mathematical equation devised by Gibb.[20]

At a given wavelength, the total optical density (TOD) is the sum of the optical densities (OD) of the two components, in this case cholic acid (c) and desoxycholic acid (d) The optical density attributable to each component may also be expressed as the product of the constant, K, which represents the optical density per unit concentration of the material added, and the concentration C, expressed here in micrograms.

$$TOD = OD_c + OD_d = K_cC_c + K_dC_d$$

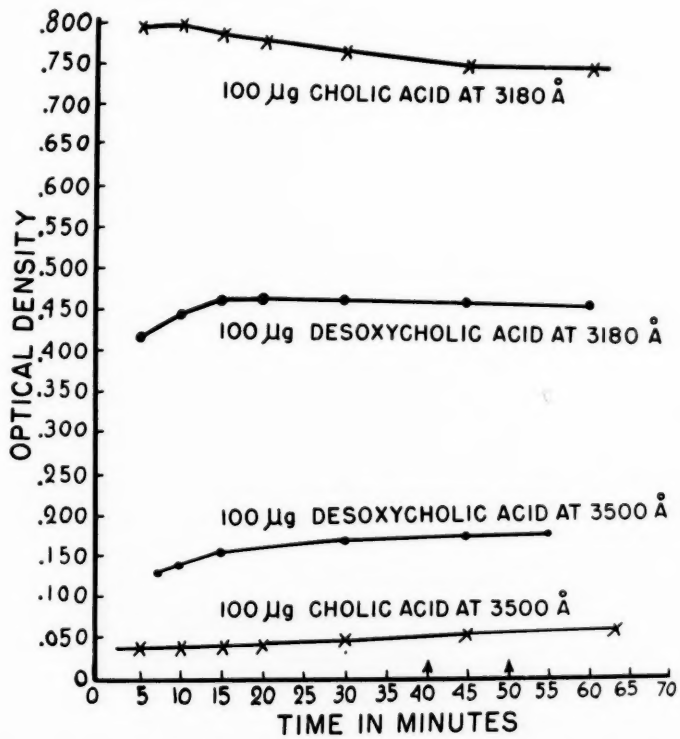


Fig. 2.—The effect of time upon the stability of the absorption of cholic acid and desoxycholic acid.

TABLE I. RESULTS OF RECOVERY STUDIES ON MIXTURES OF MICROGRAM QUANTITIES OF DESOXYCHOLIC ACID (DA) AND CHOLIC ACID (CA)

| COMBINED | RECOVERED | |
|---|---|---|
| D.A. + C.A. | D.A.* | C.A.† |
| 25 + 75 | 26 | 78 |
| 50 + 50 | 49 | 52 |
| 75 + 25 | 79 | 25 |

*Average recovery = 102 per cent.
†Average recovery = 103 per cent.

The average optical densities obtained in ten determinations of 100 microgram quantities at 3,180Å and 3,500 Å were substituted into the foregoing equation and the two following equations obtained:

(1) $C_c = \text{TOD } (3{,}180 \text{ Å}) \ 0.176 - 0.610 \text{ TOD } (3{,}500 \text{ Å})$
(2) $C_d = \text{TOD } (3{,}500 \text{ Å}) \ 0.831 - 0.0615 \text{ TOD } (3{,}180 \text{ Å})$

Application of this procedure to duplicate mixtures containing known amounts of both cholic acid and desoxycholic acid give the recoveries indicated in Table I.

## APPLICATION OF THE METHOD

One-half milliliter of drainage bile was diluted to 25 ml. in a volumetric flask with the extraction fluid (absolute alcohol, acetone, ethyl ether, 1:1:1), the whole mixture was well

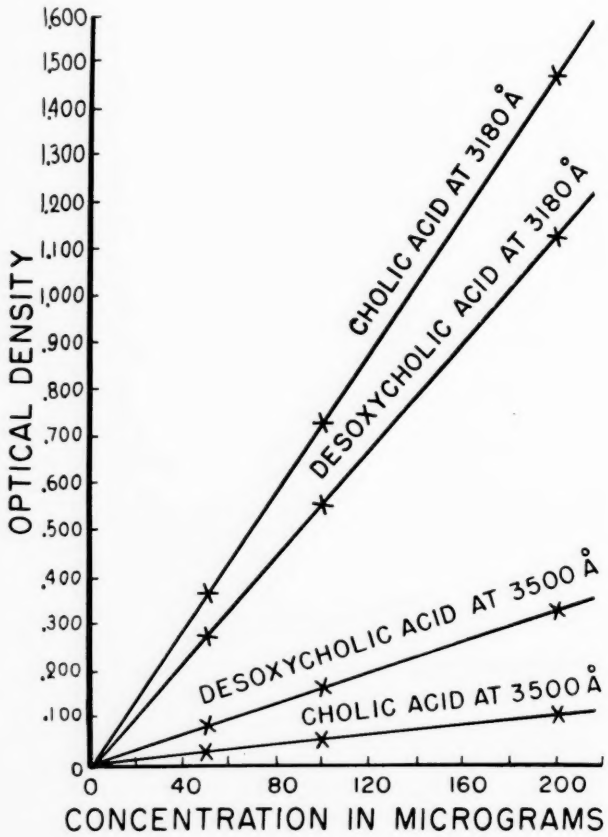


Fig. 3.—Absorption versus concentration of cholic acid and desoxycholic acid in 5 milliliters of 65 per cent sulfuric acid.

shaken, then filtered. One milliliter of the solution was pipetted into a 50 milliliter beaker and evaporated to dryness on the hot water bath, then cooled to room temperature. Five milliliters of 65 per cent sulfuric acid were added. The optical density was measured at 3,180 Å and 3,500 Å on the Beckman DU Quartz Spectrophotometer with the ultraviolet attachment. The concentrations of cholic acid and desoxycholic acid were calculated by substituting values into the equations 1 and 2 previously given.

## RESULTS

Recovery studies were carried out on seven different bile samples to which 1.25 mg. of desoxycholic acid and 2.5 mg. of cholic acid were added per milliliter of bile. These were diluted with the extracting fluid to 100 ml. in a volumetric flask. Recovery of cholic acid was 96 ± 10 per cent and desoxycholic acid 89 ± 10 per cent.

Bile was collected by T-tube from patients with biliary fistulas. Six patients were followed from nine to nineteen days. A total of 56 determinations were made in duplicate. The cholic acid concentrations were determined by the ultraviolet absorption method and compared with concentrations determined by the Reinhold and Wilson colorimetric method[9] on aliquots of bile extracted as above. The results are given in Fig. 4.

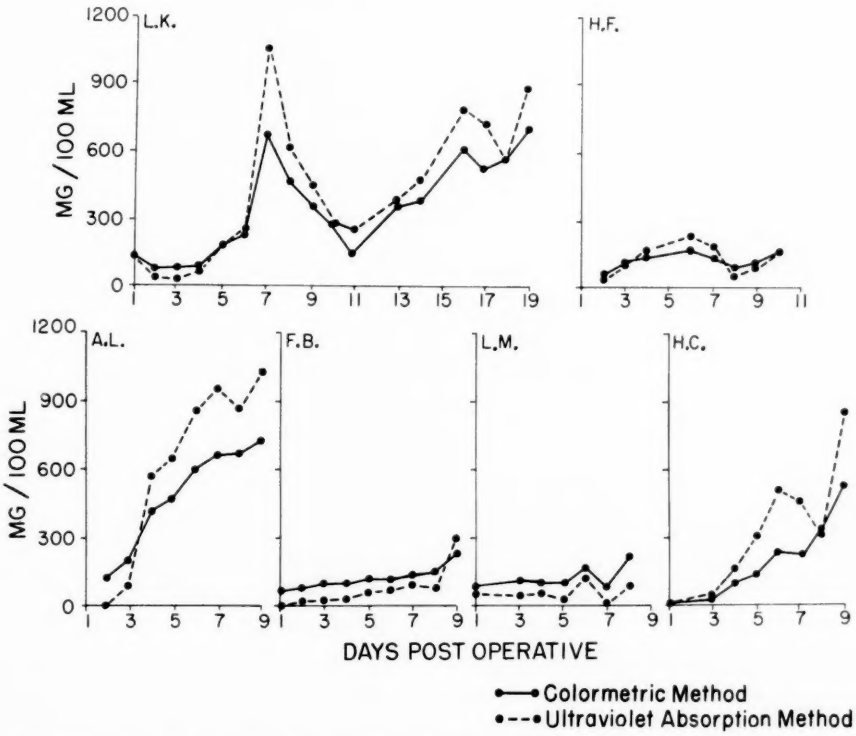


Fig. 4.—The concentration of cholic acid in human fistula bile obtained postoperatively.

Statistically, the correlation between the two methods is highly significant at the level of 1 per cent.

The peak from the sixth to the ninth day on patient L. K. occurred when the patient was fed his own bile in fruit juice.

The desoxycholic acid concentrations determined simultaneously on the same patients are graphed in Fig. 5.

The relative average percentages of the bile acids in all of the human fistula bile samples were 42 per cent desoxycholic acid and 58 per cent cholic acid.

## DISCUSSION

The highly significant correlation between the Reinhold and Wilson method[9] and the ultraviolet method for cholic acid demonstrates the validity of the ultraviolet method as specific and sensitive for cholic acid.

Fig. 4 shows agreement with other authors[21, 22] in substantiating the fact that after operation, including the establishment of a bile fistula, patients usually have a very low cholic acid production during the first three or four days. After this point the cholic acid production increased in varying degree in the different cases. This is consistent with the view that Gray and associates[23] found the recovery from inhibited production to be rapid in the first days in cases in which the liver was clinically normal, but slower when the liver had been damaged. Damaged livers did not completely regain their function of concentrating bile acids, and there was apparently a limit to the extent of recovery after injury.

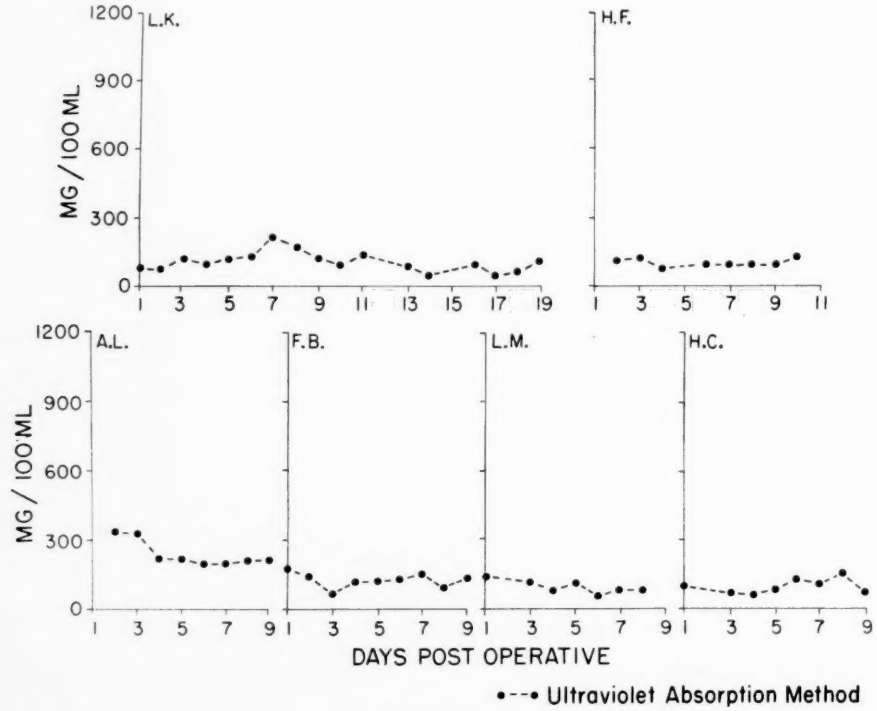


Fig. 5.—The concentration of desoxycholic acid in human fistula bile obtained postoperatively.

The percentages of 42 per cent for desoxycholic acid and 58 per cent for cholic acid approximate the values which Doubilet[24] obtained by fractional analysis.

## SUMMARY

A method has been presented for the simultaneous quantitative determination of cholic acid and desoxycholic acid. This method is based upon the ultraviolet absorption of a sulfuric acid solution. It has been applied quantitatively to human fistula bile.

Recoveries of 96 ± 10 per cent of added cholic acid and 89 ± 10 per cent of added desoxycholic acid were obtained.

Comparisons of the levels of cholic acid as determined by the ultraviolet method show a high significance (one per cent) of agreement with an established colorimetric method.

The average percentages of desoxycholic acid and cholic acid found in human fistula bile as determined by the ultraviolet absorption method support the findings that desoxycholic acid and cholic acid occur in approximately equal parts in human bile.

Cholic acid levels were low for the first three or four postoperative days, and then gradually rose.

Further clinical work on bile is in progress.

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# A METHOD FOR THE SIMULTANEOUS QUANTITATIVE DETERMINATION OF CHOLIC ACID AND DESOXYCHOLIC ACID

## II. Application to Blood

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THE information upon bile acids in blood is very limited, and results disagree widely.[1-6] Because of the inaccessability of human bile for diagnostic studies, a method for the determination of bile acids in blood should prove of value in clinical studies, diagnostic or research.

This paper outlines the application of a method for the simultaneous quantitative determination of cholic acid and desoxycholic acid to blood.

### MATERIALS AND METHODS

*Materials.*—The materials used in this experiment were essentially the same as those employed in the previous paper.[7] Normal concentration ranges of desoxycholic acid and cholic acid were determined on plasma samples from healthy, nonfasting blood donors to the blood bank at the State University of Iowa.

*Method.*—Two-tenths milliliter of plasma or serum was pipetted into an 8 ml. test tube. Five milliliters of the extracting fluid (absolute alcohol, acetone, ethyl ether, 1:1:1) were then added by burette. The mixture was thoroughly stirred. The tubes were heated in a constant temperature hot water bath at 60° C. for three minutes. They were then centrifuged and the supernatant liquid was decanted and filtered through Whatman No. 43 filter paper into a 50 ml. beaker. The precipitate was washed with two more 5 ml. portions of extracting fluid, heated, centrifuged, and filtered into the same beaker. The filtrate was then evaporated to dryness on the hot water bath. Five milliliters of 65 per cent sulfuric acid were added to the residue. The solution was read at 3,180 Å and 3,500 Å on the Beckman DU Quartz Spectrophotometer with ultraviolet attachment after standing forty to fifty minutes. The concentrations of each component were calculated as in the previous paper.[7]

### RESULTS

Recovery studies were carried out in duplicate on twenty different plasmas to which desoxycholic acid and cholic acid were added in concentrations of 50 and 100 micrograms. Recovery of cholic acid was 85 ± 5 per cent and of desoxycholic acid 77 ± 5 per cent.

Comparisons in twenty different persons showed no significant difference between desoxycholic acid concentrations in plasma and serum.

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The material for this paper was taken in part from the thesis written by Lawrence C. Kier in partial fulfillment of the requirements for the Doctor of Philosophy degree in the Graduate College, Department of Biochemistry, State University of Iowa, June, 1951.

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The average desoxycholic acid concentrations for healthy individuals' plasma were 35 ± 10 mg. per 100 ml. in 55 male subjects and 42 ± 10 mg. per 100 ml. in 42 female subjects. Plasma cholic acid was absent.

Clinical cases were selected in which it was expected that the plasma bile acid levels would be abnormal, due to parenchymal liver damage and biliary tract obstruction.[8-11]

Patients with parenchymal liver damage (cirrhosis) had low desoxycholic acid levels. In biliary obstruction cases the values for desoxycholic acid were higher than the mean values in healthy individuals (Table I).

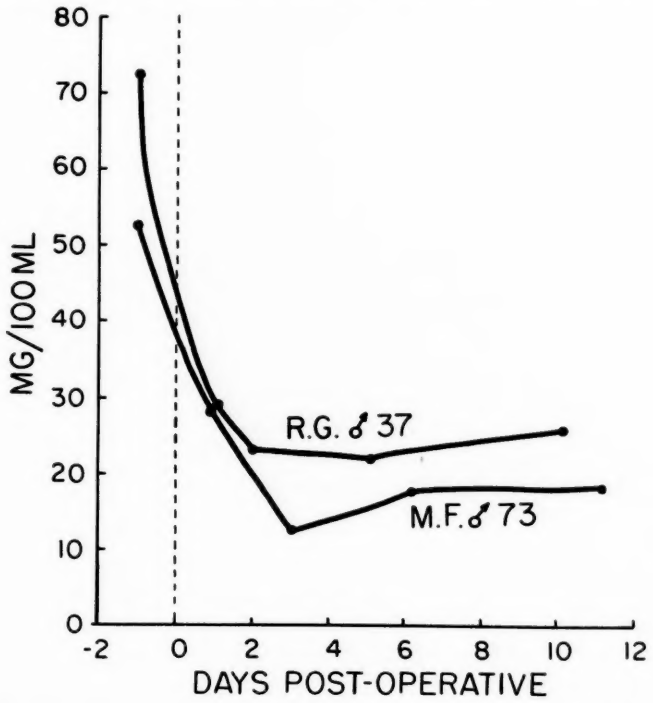


Fig. 1.—Pre- and postoperative desoxycholic acid plasma levels of biliary obstruction cases.

Plasma cholic acid was absent in clinical cases as measured by this technique.

Plasma levels of two patients with biliary tract obstruction were studied pre- and postoperatively. Surgical removal of the obstruction was followed by the reduction of desoxycholic acid levels in the plasma (Fig. 1).

TABLE I. MILLIGRAMS OF DESOXYCHOLIC ACID PER 100 ML. OF SERUM OR PLASMA FROM HEALTHY AND CLINICAL INDIVIDUALS

| TYPE | NO. OF CASES | SEX | MG./100 ML. | |
|---|---|---|---|---|
| | | | MEAN | RANGE |
| Normal | 55 | Male | 35 | 25-45 |
| Normal | 42 | Female | 42 | 32-52 |
| Cirrhosis of liver | 14 | Male | 17 | 4-28 |
| Cirrhosis of liver | 5 | Female | 21 | 16-29 |
| Biliary tract obstruction | 6 | Male | 58 | 50-78 |
| Biliary tract obstruction | 10 | Female | 87 | 53-156 |

## DISCUSSION

Figures for plasma bile acid concentrations by the different authors[1-6] are not consistent. The application of the method used was usually based upon the assumption that the bile acids in the circulation are qualitatively identical with cholic acid. Previous methods which are now conventionally accepted for cholic acid to respond to desoxycholic acid in varying degrees, from traces by the Reinhold and Wilson method to 66 per cent as much response by the Pettenkoffer reaction.

Tashiro and associates[12] contended that the blood "bile salts" cannot be identical with conjugated or free cholic acid, but rather with desoxycholic acid or its conjugates. His evidence consisted chiefly of the precipitability of the Pettenkoffer positive substance in blood by magnesium and by barium, the increase of the color reaction after treatment with chloroform, and the blue fluorescence of the Pettenkoffer-Tashiro color given by blood "bile salts" and desoxycholic acid, in contrast to the purple fluorescence given by free or conjugated cholic acid.

According to Lichtman,[13] the cholates should pass through the kidney into the urine more readily than the desoxycholates, thus causing a lower concentration in the blood of cholic acid than desoxycholic acid.

The results obtained for human plasma by the ultraviolet absorption method would seem to concur with the above; namely, that the bile acid predominating in human blood is free or conjugated desoxycholic acid. No cholic acid was detected in human blood by this technique.

The interference of other compounds in absorption measurements was considered. Reinhold and Wilson tested a large number of compounds and demonstrated that they did not interfere with their cholic acid determinations. The highly significant correlation of the two methods applied to a biological system[7] would seem to indicate that in all probability these many compounds were not interfering to any extent with the ultraviolet absorption method either. This is further substantiated by a general consideration of the ultraviolet absorption of the steroids and cholesterol. Steroids and cholesterol have a maximum absorption occurring at significantly shorter wave lengths than either cholic acid or desoxycholic acid.

The finding that patients with biliary tract obstruction had decidedly higher plasma concentrations than normal was to be expected, according to Josephson.[8] The plasma levels were immediately reduced to a normal or even below normal level when the obstruction was removed surgically and the bile acids were drained through the fistula. Cholic acid was not detected in the plasma of these patients.

The significantly lower level of desoxycholic acid in the plasma of patients with cirrhosis of the liver than in the normal subjects was anticipated, since generalized injury to the parenchyma of the liver diminishes the production of the bile acids.[9-11] Cholic acid was not detected in the plasma.

SUMMARY

The application of an ultraviolet absorption method for the simultaneous quantitative determination of cholic acid and desoxycholic acid to human blood plasma or serum has been presented.

Recoveries of 85 ± 5 per cent for cholic acid and 77 ± 5 per cent for desoxycholic acid were obtained.

The mean desoxycholic acid level was 35 mg. per 100 ml. in 55 healthy nonfasting male subjects and 42 mg. per 100 ml. in 42 healthy nonfasting female subjects.

Clinical cases with biliary tract obstruction had a mean desoxycholic acid level of 58 mg. per 100 ml. for six male patients and 87 mg. per 100 ml. for ten female patients. Clinical subjects with cirrhosis of the liver had a mean desoxycholic acid level of 17 mg. per 100 ml. for 14 male patients and 21 mg. per 100 ml. for 5 female patients. Cholic acid was not detected in human plasma or serum in either healthy or clinical subjects.

Further work on clinical cases is in progress in the hope of establishing conclusive correlations between bile acid analysis of the bile and bile acid concentrations of the blood. Such evidence would prove of value in using blood level concentrations in clinical diagnostic and prognostic studies.

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# A METHOD FOR THE PHOTOELECTRIC STUDY OF THE COAGULATION OF WHOLE BLOOD

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IN THIS article a method is described whereby a graphic record of all phases of the process of coagulation in unaltered whole blood is obtained by photoelectric means. The method is adapted to the simultaneous study of aliquots of a single sample. Provision is thus made for the study of the effect of serial concentrations of one or more introduced factors with simultaneous controls.

## THE PROBLEM

The search for a basic or fundamental coagulation time is complicated in that the latent coagulation mechanism is not activated except by a combination of many of the physical factors in the experimental environment. Control of such factors, upon which comparable results are dependent, is easily obtained in some instances such as the temperature, the size and shape of the containing vessel, and the nature of the surface of the container. The venipuncture is less easily controlled, but must be carefully standardized. The motion of tilting the tube to detect coagulation as used in the methods of Lee and White[1] and of Barker and Barker[2] has an accelerating effect on coagulation and is most difficult to control. The photoelectric principle eliminates all need for such motion.

In addition to rigid control of the various physical factors in the experimental environment, it is important to minimize or eliminate manipulations which alter the coagulation mechanism from within. The described method is adapted to the use of whole blood, and thus eliminates the need for anticoagulants and centrifugation.

The photoelectric principle for the determination of blood coagulation was first used in a preliminary way by Festen[3] with whole blood, and was further developed by Nygaard[4] using recalcified plasma. The major advantage of the photoelectric principle over those used by earlier workers is that this principle lends itself to a graphic recording of all phases of coagulation. It

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The guidance of Dr. Howard L. Alt in pursuing this study is most gratefully acknowledged.

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not only shows the start, rate, and completion of coagulation, but indicates the amount of fibrin formed as well. One or all of these factors may be of importance in any one case, and cases are shown herein which would be entirely missed by methods showing only the start of coagulation. Nygaard's method shows all phases of coagulation, but is physically unsuited for the use of whole blood and for multiple simultaneous determinations.

In studying coagulation problems, it frequently is desirable to add small increments of such substances as heparin, protamine, thromboplastin, or the like to serial samples of blood from the same individual. Such is the basis of both prothrombin time and heparin titration tests. The photoelectric method has been applied to prothrombin time determinations by Losner, Volk, Jacobi,

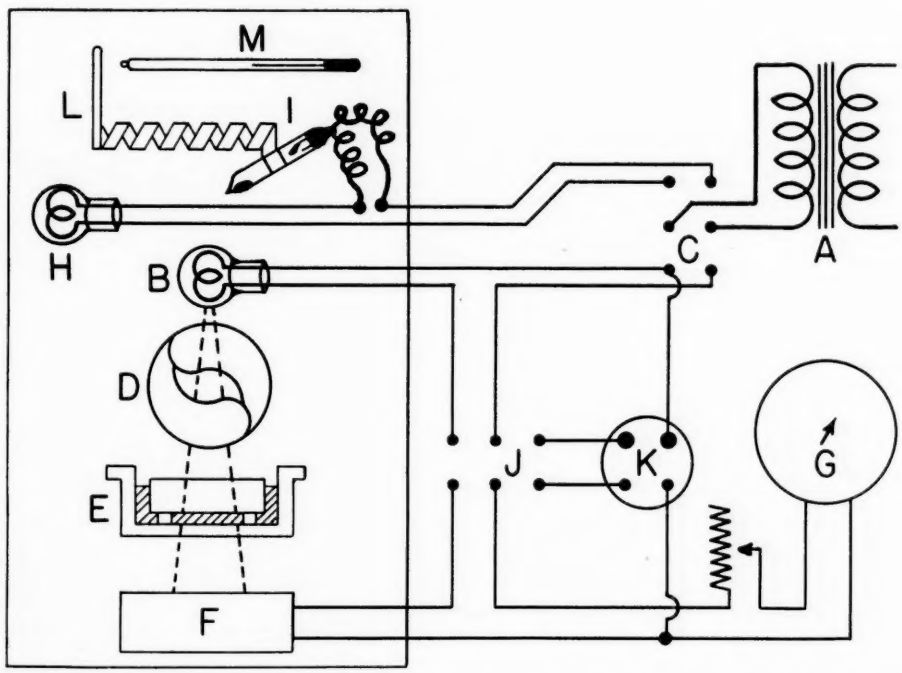


Fig. 1.—Diagram of the coagelometer: *A*, transformer; *B*, exciter bulb; *C*, selector switch; *D*, iris diaphragm; *E*, cuvette; *F*, photoelectric cell; *G*, micro-ammeter; *H*, heater bulb; *I*, thermostat; *J* and *K*, switch and socket for accessory units; *L*, heater control lever; *M*, thermometer.

and Newhouse.[5] The present method, since it is especially adapted to the use of whole blood, is valuable in giving an exact end point in heparin titration tests. The ability to study simultaneously aliquots of a single sample aids in avoiding fluctuations due to variables (primarily in the venipuncture).

## THE METHOD

Principles of the apparatus include a vertical optical axis, a plastic cuvette with a blood film of fixed thickness, and direct measurement of the amount of

light reaching the blocking layer photoelectric cell. Temperature is controlled throughout. A plotted curve shows the beginning, rate, and completion of fibrin formation.

The coagelometer as developed for this method is shown schematically in Fig. 1. The device consists of a heavy duty line transformer (*A*) connected to the 32 candle power automobile headlight bulb (*B*) by way of a double-pole-double-throw switch (*C*). Control of the amount of light is by the iris diaphragm (*D*).

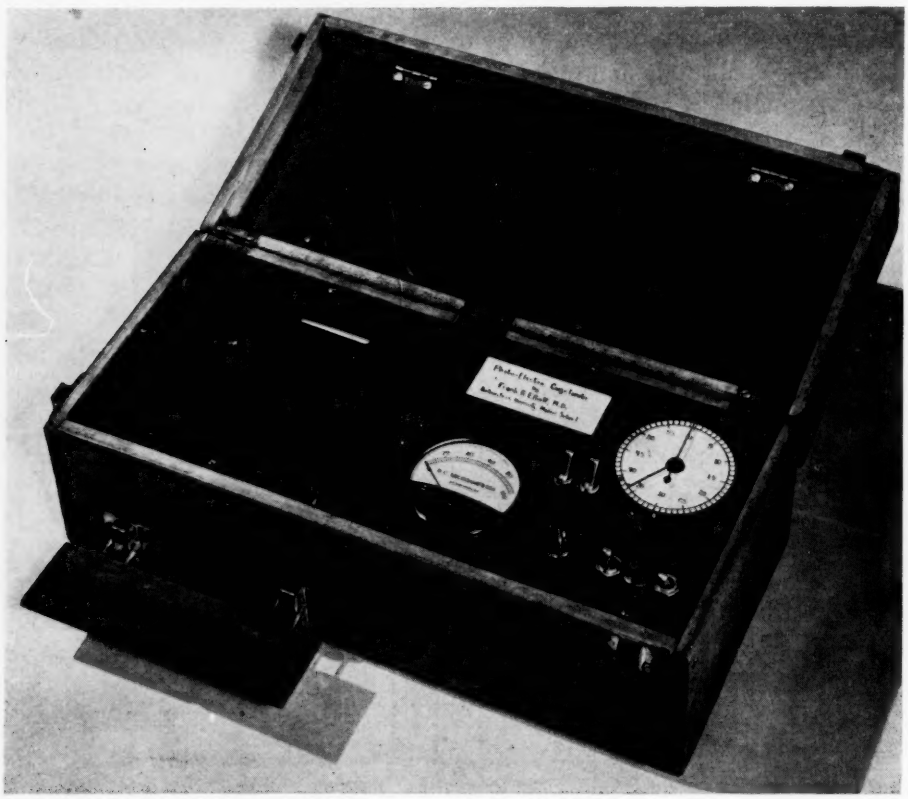

Fig. 2.—Photograph of the coagelometer.

The optical axis of the machine is placed in the vertical axis to minimize the effects of erythrocyte sedimentation. The blood sample is contained in the polysterine cuvette (*E*) which is shown in transverse sectional view. The cuvette is made from a simple tray, manufactured by the Celluplastic Corporation for E. R. Squibb and Sons and used by them for distribution of drug samples. The blood film is maintained at uniform thickness by being contained under cover-slides of one-fourth inch Plexiglas elevated on 0.5 mm. legs (punchings of plastic of this thickness) which are cemented to the cover slides.

It was found that the variation in light was due not only to changes in light transmission, but also to changes in light scatter. The apparatus is so designed that the amplitude of curve derived from each factor is at a maximum. The photocell circuit consists of a blocking layer type of cell (*F*) wired directly to a 0 to 100 microammeter (*G*). It will be noted that a potentiometer is placed in this circuit. This is used only for protection of the meter

while selecting filters (placed directly under the cuvette) when setting up for determinations on very anemic blood or on recalcified plasma. The zero position of this potentiometer must be electrically stable.

The "off" position of the double-pole-double-throw switch (*C*) supplies power for a heater bulb (*H*) controlled by the bimetallic coil and mercury switch of a household furnace stack control (*I*). It will be seen that illumination from this bulb cannot interfere with actual readings, since the heater bulb is not on at the same time as a reading is taken.

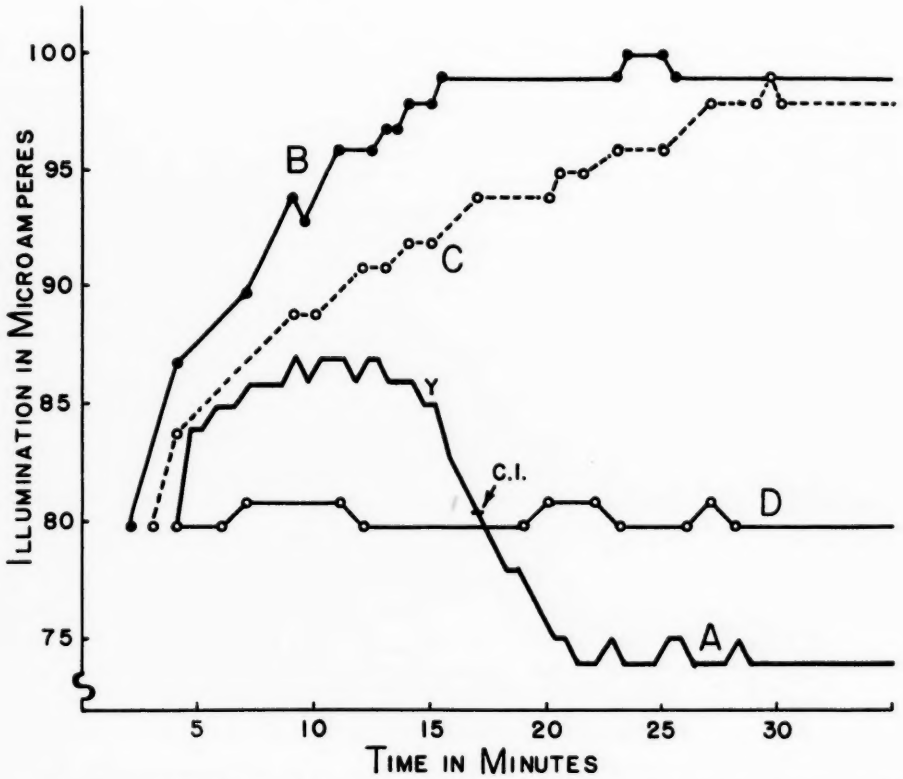


Fig. 3.—Curve *A* is a typical normal coagulation curve showing the coagulation index (*C.I.*) and the approximated end point of the Lee and White method (*Y*). Other curves shown are those of oxalated whole blood (*B*), heparinized whole blood (*C*), and heparinized plasma (*D*).

In the apparatus, the light bulb, iris diaphragm, cuvette, and photoelectric cell are actually in triplicate, selected by a double-pole-triple-throw switch to allow runs to be made in triplicate. An additional double-pole-double-throw switch (*J*) connected to a four pin socket (*K*) provides for a bank of ten additional lamp-cuvette-photocell-heater units to be plugged in when additional parallel studies are desired. The lever for setting the thermostat (*L*) and the built-in thermometer (*M*) are also indicated, while the electric timer and line switches for the timer and power transformer are shown only in the photograph (Fig. 2). There are storage spaces in the lid so that the equipment is self contained and easily portable.

The apparatus is set up in any convenient space reasonably close to the patient (see below), plugged into the 115 volt line, and allowed to reach thermal equilibrium at 37° C. Blood is obtained from the anticubital vein through as clean a puncture as possible. Twenty-gauge needles and clean, dry syringes are used. Blood is drawn slowly into a small syringe for

one minute after the tourniquet is released. Syringes are then changed without pressing on the vein or moving the needle. The first specimen is discarded (or used for other purposes), and the time is taken from the first appearance of blood in the second syringe. After the required amount of blood is drawn as promptly as possible without pulling back on the plunger with any appreciable force, the blood is transferred in 2.5 ml. lots to as many cuvettes as may be needed. This initial transfer must be made promptly, but one may then take two or three minutes to transport the cuvettes to the machine. The floor or office laboratory was used for the clinical cases studied herein.

As soon as the cuvettes can be placed in their respective cells, the illumination of each cell is set at the arbitrarily selected value of 80 microamperes. Readings are started at four minutes, and continued at half minute intervals until the curve of coagulation has been completed and a base line established.

## RESULTS

*The Normal Curve.*—In Nygaard's curve there was a preliminary period of no change in light transmission, followed by a period of decrease in light transmission of gradual onset, reaching a phase of maximum rate of change, and a gradual leveling off to a flat phase. The curve produced by the present method

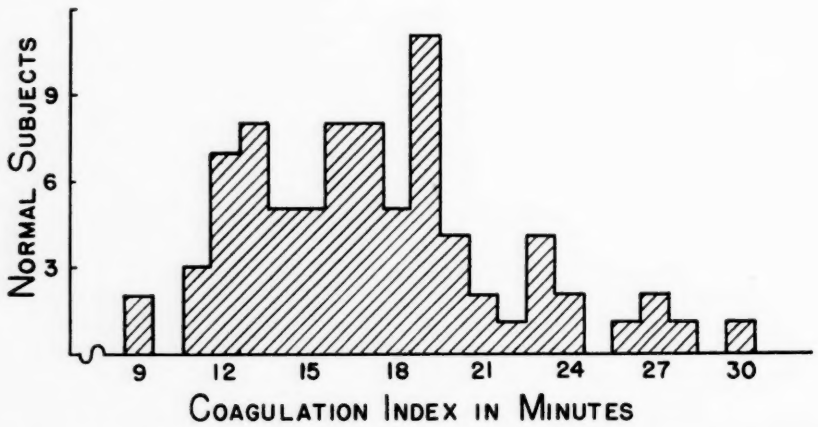


Fig. 4.—Distribution curve of the coagulation index in eighty normal individuals.

(Fig. 3, curve A) differs from this only in that the initial phase shows increasing light transmission. This initial rise with whole blood is due to sedimentation of the formed elements. When coagulation is prevented by heparin or oxalate (Fig. 3), this initial rise continues until sedimentation is complete, while no such rise occurs when the formed elements of the blood are removed.

With the exception of the initial rise, the curve of the process of coagulation was found to be entirely similar to that of Nygaard. By teasing with a fine wire, it was found on numerous determinations that the first fibrin threads could be drawn from the specimen at the peak of the curve, while a surface clot similar to that constituting the end point of the Lee and White method (as done by observation of a free sample of the same specimen in an identical plastic tray) was obtained early in the decline of the curve (at approximately point *Y*, Fig. 3).

For purposes of comparison, the geometrically well defined mid-point in amplitude of the curve (where it is transected by a horizontal line midway between its maximum and minimum values) has been selected as an end point. Nygaard named this the point of maximum velocity of fibrin formation (PMFF), but, since this point is determined on a geometric basis, an abstract term is to be preferred. The time relation of this point is herein referred to as the coagulation index, since the term "coagulation time" implies the use of the method of Lee and White.

As an indication of the accuracy of this method, determinations of the coagulation index of ten portions of a single specimen of whole blood were made simultaneously. These averaged thirteen minutes, with a standard deviation of 0.5, or an expected range of from twelve to fourteen minutes and a coefficient of variation of 4 per cent. The distribution curve of the coagulation index in eighty normal individuals is shown (Fig. 4). The standard deviation is 4.7 minutes, with a mean value of seventeen minutes.

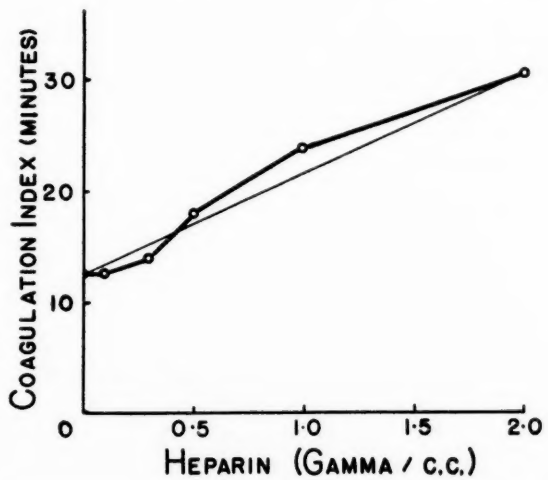


Fig. 5.—Demonstration of linear correlation obtained through simultaneous coagulation index determinations on portions of the same sample of normal blood to which increasing amounts of heparin have been added.

Fig. 5 shows simultaneous coagulation index determinations on portions of the same sample of normal blood to which small increasing amounts of heparin have been added. The low coefficient of variation gives good linear correlation. This demonstrates the value of this method in the study of the various facets of the coagulation mechanism.

## ABNORMAL CURVES

Clinical application of this method produces very precise information as to the onset, rate, and completion of fibrin formation. It is well known that the rate of fibrin formation is altered in certain hemorrhagic diseases, and some abnormal types of coagulation curve are demonstrated by the cases reported.

CASE REPORTS

CASE 1.—R. G., a 34-year-old white woman without a family history of bleeding, had a history of easy bruising for eight weeks, menorrhagia with her last menstrual period, and ecchymoses and repeated epistaxis during the nine days prior to admission. The capillary fragility test was positive, the platelet count was 3,000 per cubic millimeter, and the bone marrow showed an increase in megakaryocytes. The Duke bleeding time was seven minutes.

CASE 2.—M. B., a 64-year-old white woman, had undergone splenectomy fifteen years previously for primary thrombocytopenic purpura. The platelets rose to 130,000 postoperatively, but subsequently dropped to the preoperative level. At the time of this study the platelet count was 5,860 per cubic millimeter, bleeding time thirty minutes, Lee and White coagulation time four minutes, capillary fragility increased, and there were numerous ecchymoses and petechial hemorrhages.

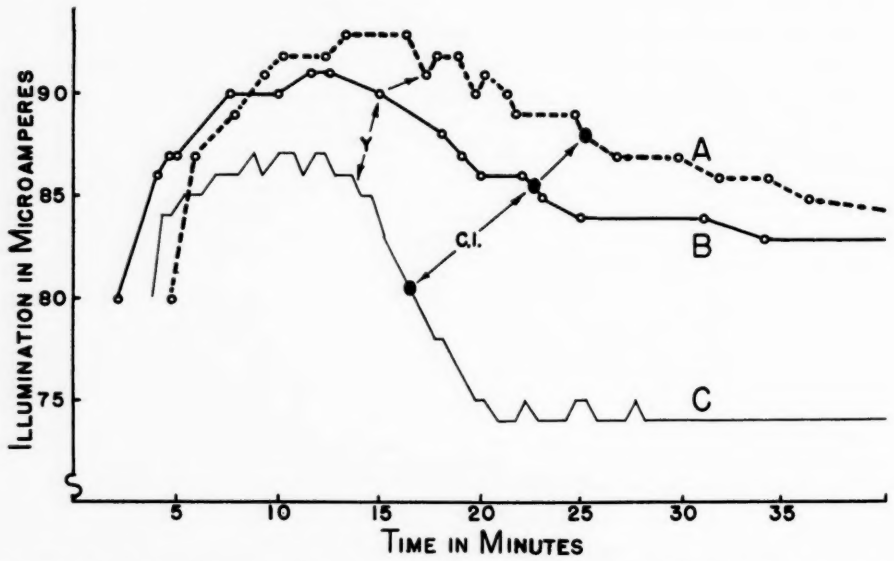


Fig. 6.—Coagulation curves in thrombocytopenic purpura showing the coagulation index (*C.I.*) and the approximated end point of the Lee and White method (*Y*): *A*, Case 1; *B*, Case 2; *C*, a typical normal curve.

The coagulation curves in these cases are typical of thrombocytopenic purpura. It is seen that there is the usual initial rise, that the first fibrin formation occurs within normal limits of time, and that fibrin formation is both slow and scant, clot formation not being completed until after forty minutes. It is further noted that the approximated point Y at which the Lee and White coagulation time is read falls within normal limits.

CASE 3.—P. McC., a 35-year-old white man, first had a bleeding tendency at 3 months of age, with severe bleeding episodes at intervals of six to eighteen months since that time. During one of these episodes the platelet count was 185,600 per cubic millimeter, the prothrombin (Quick one-stage) 98.1 per cent, the fibrinogen 140 mg. per 100 ml., the Duke bleeding time two minutes, and the Lee and White coagulation time sixty minutes.

This case is typical of hemophilia, with the exception that no family history of bleeding tendency can be obtained. Due to delayed coagulation, it

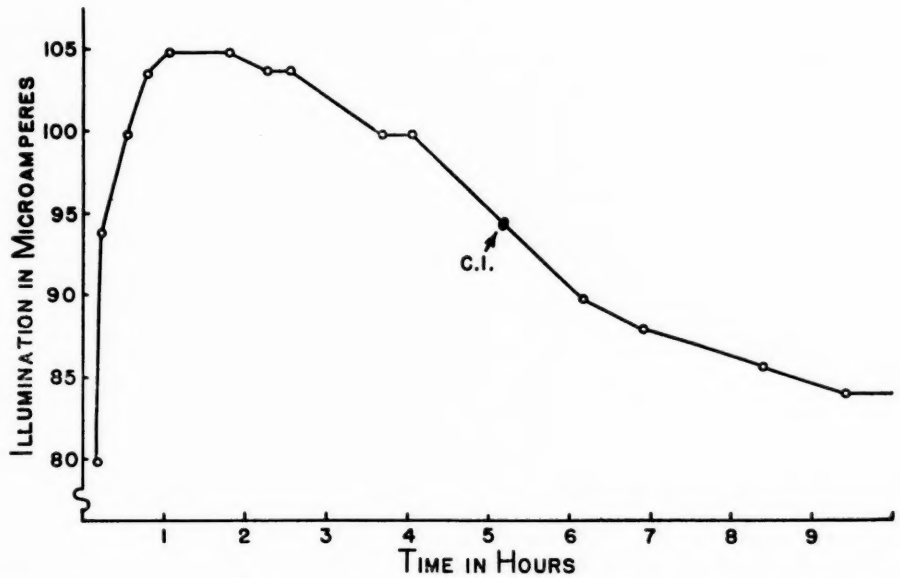


Fig. 7.—Coagulation curve in hemophilia (Case 3) with the coagulation index indicated (*C.I.*).

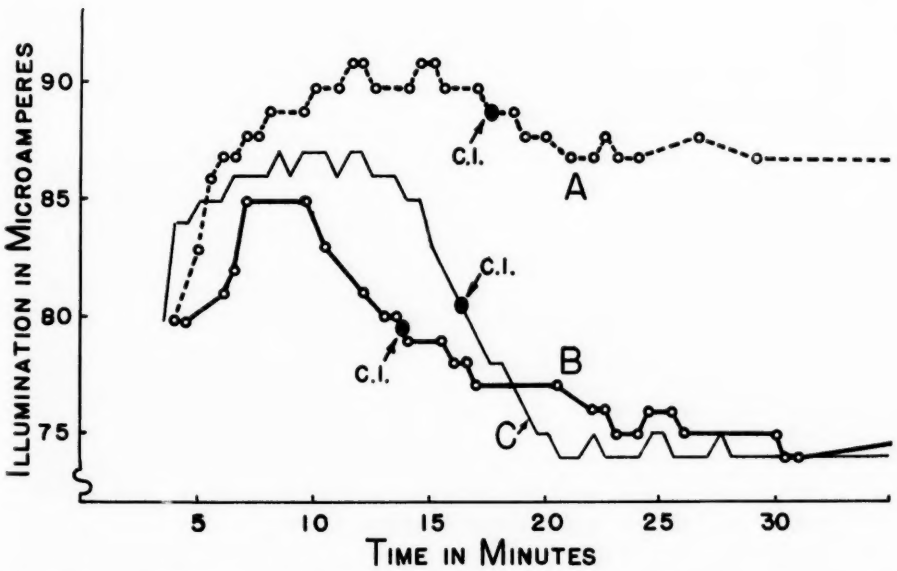


Fig. 8.—Coagulation curves in Case 4 hypofibrinogenemia (*A*), Case 5 pseudohemophilia (*B*), and a typical normal subject (*C*) with the coagulation index indicated (*C.I.*).

is necessary to plot the abscissa in terms of hours. It is seen that the initial rise in this curve is as extreme as that of heparinized or oxalate blood, where sedimentation of the formed elements is allowed to progress to completion. Following this rise there is an additional flat latent period before fibrin formation starts. Once started, fibrin formation continues for seven hours, giving a coagulation index of five hours and ten minutes.

CASE 4.—M. S., a 73-year-old white woman with myelomonocytic leukemia, developed spontaneous ecchymoses and repeated epistaxis. The platelet count was 144,780 per cubic millimeter, the prothrombin (Quick one-stage) 74.7 per cent, the Ivy bleeding time forty-five seconds, and the Duke bleeding time two minutes. The blood fibrinogen varied from 59 to 89 mg. per 100 ml. The Lee and White coagulation time was unsatisfactory in that insufficient fibrin was formed to give a discernible clot.

This curve of markedly low amplitude is typical of cases with a quantitative deficiency of fibrinogen. The curve is normal to the onset of fibrin formation. Fibrin formation starts, but almost immediately ceases as all the fibrinogen is converted to fibrin. Nygaard made similar observations and established that the measurement of amplitude of the coagulation curve is a quantitative determination of fibrin.

CASE 5.—J. R., a 51-year-old white man, first showed a bleeding tendency at the age of 7 years. The platelet count was 150,000 per cubic millimeter. Repeated coagulation time determinations were within normal limits, and the clot retraction was normal. The Duke bleeding time was twenty-seven minutes. The capillary fragility was normal. There was a family history of eight known cases of a similar bleeding tendency.

The above case shows a normal coagulation curve. This is consistent with Macfarlane's[6] theory that pseudohemophilia is due to changes in the vascular bed without there being any alteration in the coagulation mechanism.

## SUMMARY AND CONCLUSIONS

A photoelectric method for the study of the coagulation of unaltered whole blood has been described. The method measures the beginning, rate, and completion of fibrin formation. For comparative purposes, the mid-point on the coagulation curve has been designated as the coagulation index.

The main advantages of this method over the Lee and White method are that (1) it gives a well defined end point, (2) it measures the entire process of blood coagulation, (3) agitation of the blood due to tilting of the tube is eliminated, and (4) progressive formation of fibrin may be graphically recorded.

The photoelectric coagelometer has been adapted for the simultaneous study of ten aliquots of a single blood sample. This provides a useful experimental set-up for studying the effects of varying concentrations of coagulants and anticoagulants on blood clotting.

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# PROCEEDINGS OF THE CENTRAL SOCIETY FOR CLINICAL RESEARCH

*Twenty-Fifth Annual Meeting*

*Chicago, Ill., Nov. 7 and 8, 1952*

## ABSTRACTS

### 1. DIAGNOSTIC USE OF RADIOACTIVE IODINE: REPORT OF 1000 CASES

EDWIN C. ALBRIGHT, M.D. (BY INVITATION), AND EDGAR S. GORDON, M.D., MADISON, WIS.

The objective estimation of thyroid activity is a necessity in many clinical problems. The diagnostic use of radioactive iodine for this purpose has been studied in 1000 patients in the past five years, and the information gained permits evaluation of this procedure.

Indications for its use are: (a) to establish hyper- or hypothyroidism in the presence of diseases which may mask thyroid dysfunction, (b) to establish normal thyroid function in the presence of diseases which may mimic thyroid dysfunction, (c) to establish the identity of metastatic lesions, and (d) for evaluation of patients for radioiodine therapy.

The twenty-four hour $I^{131}$ accumulation as a measure of thyroid activity is proportional to thyroid hormone synthesis and release, with certain well-recognized exceptions. The exceptions which may result in a low $I^{131}$ uptake are thyroid saturation with $I^{127}$, as from Lugol's solution, contrast x-ray media, topical iodine application, desiccated thyroid; and concomitant ingestion of thiouracil drugs. High uptakes may be due to thyroid hyperplasia induced by prolonged use of thiouracil drugs or to chronic iodine deficiency. Return of the uptake to normal following thiouracil induced hyperplasia has been found to require from two to six months after withdrawal of the drug. Extremely high uptakes were found in several members of one family with goiters who had been on iodine-deficient diets.

The measurement of twenty-four hour $I^{131}$ accumulation is believed to be preferable to determination of accumulation rate, discharge rate, and urinary excretion for routine clinical use, as it is accurate, reproducible, and the least complicated. The range of uptake values observed are for the hypothyroid 0 to 9 per cent, the euthyroid 10 to 40 per cent, and the hyperthyroid 50 to 90 per cent. Unavoidable overlapping in the 40 to 50 per cent zone may occur occasionally, requiring resolution of the diagnostic problem by other procedures.

### 2. TRENDS IN MITRAL VALVULOPLASTY

BERNARD A. BERCU, M.D. (BY INVITATION), EDWARD MASSIE, M.D., THOMAS H. BURFORD, M.D. (BY INVITATION), AND WITH THE TECHNICAL ASSISTANCE OF DOROTHY HOLLANDER, B.S. (BY INVITATION), ST. LOUIS, MO.

Experiences with 21 patients undergoing mitral valvuloplasty have been reviewed to determine if there has been any change in the selection or handling of patients. The severity of the disease was classified into four groups accord-

ing to the criteria of Dexter. One patient was in Group II, 15 in Group III, and 5 in Group IV. Three deaths occurred, and they were all in Group IV patients. The procedure of "finger fracture" of the valve was found to be adequate in producing a satisfactory valve orifice.

Originally it was hoped that, with operation, the conversion of patients with auricular fibrillation to a normal sinus rhythm would be facilitated by the subsequent improvement in cardiovascular dynamics. However, of 12 patients with normal sinus rhythm prior to valvuloplasty, 5 developed auricular fibrillation in the early postoperative period, and in 2 reversion to a sinus rhythm was not possible. No case of auricular fibrillation before operation developed a sinus rhythm after the procedure.

Six patients had multiple peripheral emboli prior to operation. In none of the entire group of patients were any peripheral embolic manifestations encountered during or following surgery. This has influenced us to consider the occurrence of multiple peripheral emboli a strong point in favor of doing the procedure.

Acute rheumatic fever developed postoperatively in three patients. We now routinely give penicillin before, during, and for several months after the procedure for prophylactic reasons.

Many drugs have been advocated for the changes in cardiac rate and rhythm during mitral valvuloplasty. It has been our experience, however, that cardiac medication during the procedure is usually not needed, and actually the operative course has been smoother without such drugs.

Criteria for selection of patients have not changed significantly. It seems worthwhile emphasizing, however, that the presence of auricular fibrillation, peripheral emboli, hemoptysis, and cardiac decompensation not only do not contraindicate the operation, but rather, by calling attention to the precarious state of the patient, make consideration for operation more imperative. Recent experiences with two Group IV patients who have been rehabilitated from a bedridden critical existence have called for consideration of this group for operative treatment.

## 3. USE OF AN ANION-CATION EXCHANGE RESIN AND A LIBERAL DIET IN DECOMPENSATED HEPATIC CIRRHOSIS

MAURICE M. BEST, M.D., LOUISVILLE, KY.

(INTRODUCED BY J. MURRAY KINSMAN, M.D.)

Sodium restriction is an effective means of controlling the formation of ascites and edema occurring in cirrhosis of the liver. It is often difficult to maintain patient cooperation following the prolonged use of the low-sodium diet. Several investigators have reported favorable results using a restricted sodium diet and cation exchange resins. It was considered pertinent to study the prolonged administration of a cation exchange resin and a diet restricted only by the elimination of alcohol.

Nine patients were selected on the basis that they had been forming ascites steadily for at least six months requiring repeated paracentesis and mercurial diuretics. Needle biopsy of the liver revealed presence of portal cirrhosis in all patients. Maximal formation of ascites was indicated in most patients by a very low 24-hour urine sodium excretion. An anion-cation exchange resin mixture composed of 59 per cent carboxylic acid resin, 29 per cent potassium salt of carboxylic acid resin, and 12 per cent alkyline polyamine resin was administered to a total daily dose of 60 to 70 Gm.

Repeated determinations of serum sodium, potassium, and calcium revealed no significant derangement during the period of study. A mild metabolic acidosis as indicated by lowering of serum pH $CO_2$ content and elevation of serum chloride levels was frequently noted. The acidosis was not accompanied by clinical symptoms.

Two patients died during the period of study (esophageal and postoperative hemorrhage). Four patients required continuous administration (eight to eleven months) of the resin to remain free of ascites; however, these patients are clinically asymptomatic and have required no additional paracentesis. In two patients the ascites has remained completely reabsorbed for six months following the completion of the diet and resin therapy. One patient required the use of a mercurial diuretic in conjunction with the resin for control of ascites.

Serial liver function tests were performed at intervals throughout the study. 72.2 per cent of these tests were abnormal prior to treatment as compared to 20 per cent abnormal tests at completion of the study. Serial needle biopsies of the liver revealed no significant changes except for some decrease in intrahepatic fat.

## 4. AGE, SEX, RACE, AND HEMATOLOGIC CLASSIFICATION OF 916 LEUKEMIA CASES

WILLIAM R. BEST, M.D. (BY INVITATION), AND LOUIS R. LIMARZI, M.D.,
CHICAGO, ILL.

At the University of Illinois clinics and in consultation, 916 cases of leukemia have been seen during the past twenty-six years. Approximately 95 per cent of these were observed during the past seventeen years. Ages of onset ranged from newborn infant to 82 years. When the distribution of these cases is plotted as percentage of case onsets per year of age, a sharp peak of occurrence due to acute unclassified leukemia is noted between 2 and 6 years of age. There is a low incidence during adolescence and a gradual rise to a second peak at about 55 years, due principally to the chronic leukemias. The peak of occurrence of chronic lymphocytic leukemia occurred at about 55 years; of chronic granulocytic, 45 years; and of acute granulocytic, 35 years. The less common types were particularly evident between 30 and 60 years of age.

Males accounted for 58.7 per cent of cases. The percentage of males according to different leukemic types are: chronic lymphocytic, 65 per cent; chronic granulocytic, 51 per cent; acute unclassified, 63 per cent; monocytic, 49 per cent; acute granulocytic, 51 per cent; and miscellaneous types, 65 per cent.

There were 4.2 per cent Negroes in this series. During a fifteen-year interval studied in detail, 0.26 per cent of new white clinic patients had leukemia, while only 0.04 per cent of new Negro clinic patients had this disease. The distribution of cases as to sex, age, and leukemic type is approximately the same in the Negro as in the white.

The hematologic classification used and percentage of all cases occurring in each group are: *lymphocytic, 37.4 per cent* (6.2 per cent acute, 0.9 per cent subacute, 30.4 per cent chronic); *granulocytic, 39.8 per cent* (13.2 per cent acute, 3.3 per cent subacute, 19.3 per cent chronic phase of chronic, 3.9 per cent acute phase of chronic); *acute unclassified, 11.5 per cent; monocytic, 4.3 per cent* (2.1 per cent granulocytic type, 2.2 per cent reticulum-cell type); *reticulum-cell leukemia, 4.5 per cent* (1.3 per cent undifferentiated, 1.5 per cent granulocytic type, 0.8 per cent lymphocytic type, 0.9 per cent erythrocytic type), *leukemic phase of lymphocytic sarcoma, 2.3 per cent* (1.3 per cent acute, 0.1 per cent subacute, 0.9 per cent chronic); and *plasmocytic, 0.3 per cent.* In addition 23 cases of myeloid metaplasia of the spleen were seen during this time.

## 5. MORPHOLOGIC AND METABOLIC STUDIES IN A CASE OF COEXISTENT CHRONIC LYMPHATIC LEUKEMIA AND POLYCYTHEMIA (PRIMARY?) WITH PARTICULAR REFERENCE TO UNUSUAL IRON METABOLISM

WILLIAM F. BETHARD, M.D. (BY INVITATION), MATTHEW BLOCK, PH.D., M.D., AND MELBA ROBSON, M.S. (BY INVITATION), CHICAGO, ILL.

An 82-year-old white woman was seen because of an elevated white count and extreme elevation of erythrocytes and hemoglobin. The morphology of the bone marrow, liver, and spleen was typical of chronic lymphatic leukemia, there not being any erythroblasts in the liver or spleen, and a greatly reduced number in the marrow. Polycythemia was confirmed by blood volume determination, and the arterial oxygen saturation was normal.

Plasma iron clearance rate and red cell iron uptake were determined with $Fe^{59}$. The former, when plotted semi-logarithmically, yielded two components instead of the usual one; half-times of these were 0.3 and 10.2 hours, respectively. In the latter, 99 per cent of the radioiron had appeared in the erythrocytes nine days after injection. Four serum iron determinations ranged from 7 to 10 gamma per cent, and with each determination multiple controls were performed without evidence of technical error. The serum iron binding capacity was correspondingly high (348 gamma per cent).

The data suggest that plasma beta-1 globulin cannot be entirely cleared of conjugated iron by erythropoietic tissue. The slow component of the plasma iron clearance approximates that seen in refractory anemias and may represent a process related to extra-hematopoietic utilization of iron. This phenomenon is probably normally masked, and is seen here only by virtue of the extremely low serum iron level. Attention is called to the fact that despite an increased red cell circulating mass there was morphologic evidence of a decrease in marrow erythroblastic activity and an absence of such activity in the liver and the spleen. Studies of the morphology and of the iron metabolism in an unusual case of concomitant polycythemia and chronic lymphatic leukemia are presented.

## 6. A CLINICAL STUDY OF MENINGITIS FOLLOWING PROCEDURES ON THE CENTRAL NERVOUS SYSTEM

J. PARK BIEHL, M.D. (BY INVITATION), AND MORTON HAMBURGER, M.D., CINCINNATI, OHIO

Six cases of meningitis secondary to procedures on the central nervous system have been observed recently at the Cincinnati General and Children's Hospitals. The first three (C. G. H.) followed continuous spinal anesthesia given through a plastic catheter, and were not recognized for five, eight, and twelve days thereafter. The fourth case (Ch. H.) followed infection of a surgical wound after an operation for a meningocele. The fifth (C. G. H.) followed manual withdrawal of a polyvinyl catheter, which had been inserted into the lumbar subarachnoid space seven days previously, and the sixth (C. G. H.) occurred during a course of daily streptomycin administration through a similarly placed catheter for tuberculous meningitis.

The causative agent in five cases was *Pseudomonas aeruginosa (B. pyocyaneus)*, and one a species of Eberthella *(E. dubya)* ordinarily not considered a human pathogen. Organisms isolated from all six cases were resistant to streptomycin, aureomycin, and terramycin in vitro. Three of the Pseudomonas strains that were so tested were found to be sensitive to polymyxin B; the other two were not tested. The Eberthella isolated from the fifth case was found to

be inhibited by 31 μg per milliliter of chloramphenicol. In vitro testing for possible synergism between antibiotics, using a modification of the method of Jawetz, indicated a suggestion of synergism between chloramphenicol and polymyxin B upon this organism.

The first three patients received various combinations of streptomycin, aureomycin, penicillin, and sulfadiazine, and all died. Cases 4, 5, and 6 were treated with polymyxin B and made complete recoveries from the gram-negative rod meningitis. The fourth was given polymyxin B intramuscularly and intrathecally. The fifth patient received chloramphenicol intramuscularly and polymyxin B intrathecally. The sixth received polymyxin B intrathecally along with the pre-existing dose of streptomycin intramuscularly and intrathecally, and is living with evidence only of the subsiding tuberculous meningitis.

The clinical course of *Pseudomonas meningitis* may be insidious and deceptive. The patient may show no specific signs of meningitis for as long as two or three weeks. During this phase, the disease may be detected only by careful observation, and by examination of the spinal fluid. The effectiveness of polymyxin B in combating Pyocyaneus is further demonstrated by these cases.

## 7. THE VALUE OF NEEDLE BIOPSY IN THE CHEMICAL ESTIMATION OF LIVER LIPIDS IN MAN

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Methods have been developed for the chemical analysis of lipids in specimens of liver obtained by needle biopsy. The value of these analyses depends on the extent to which a specimen of liver weighing 5 to 8 mg. can be considered representative of the whole liver, in respect of lipid content. Using a Vim-Silverman needle, 18 specimens have been taken from different sites in each of 25 post-mortem livers (13 nonfatty and 12 fatty livers) and have been examined histologically and chemically for lipids.

Histologic examination has shown that the distribution of fat throughout the human liver is relatively uniform. Chemical analysis indicated some variation in the lipid content of the specimens obtained from any one liver. The variance tended to increase with the lipid content and was slightly greater in cirrhotic than noncirrhotic livers. In no instance, however, was there evidence of a grossly uneven distribution of lipids in the liver, nor of nonfatty tissue existing simultaneously with fatty tissue in the same liver.

From the data obtained it has been possible to calculate the approximate limits of error in estimating the lipid content of the whole liver from analysis of a single biopsy specimen. Calculations have also been made to determine in a repeat biopsy the change necessary to indicate a significant change in the lipid content of the whole liver.

## 8. PAPER CHROMATOGRAPHY OF THE "PREGNANEDIOL" FRACTION OF URINE

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(INTRODUCED BY F. W. HOFFBAUER, M.D.)

Mixtures of pregnane-3,20-diol and pregnane-3,17,20-triol can be separated on propyleneglycol treated filter paper by development with propyleneglycol

saturated toluene. The steroids can be located on the paper by treatment with either a solution of antimony trichloride in chloroform or a solution of zinc chloride in glacial acetic acid.

These techniques of paper chromatography were applied to a study of the "pregnanediol" fraction of urine. The latter was obtained by incubating the urine with glucuronidase, followed by toluene extraction, and finally precipitation from alcoholic solution with sodium hydroxide. Aliquots of the precipitate were used for paper chromatography and for quantitative colorimetric assay with the sulfuric acid reaction.

The "pregnanediol" fraction of eighteen 24-hour urine specimens obtained from nine women was investigated. Two of the subjects were studied at various phases of the menstrual cycle, six were at various stages of pregnancy, and one had been ovariectomized. Paper chromatography showed that the "pregnanediol" fraction consisted of at least two components in most instances. One of the components traveled with an Rf value and produced color reactions which were practically identical with pregnane-3,20-diol, while the characteristics of the second component were practically identical with pregnane-3,17,20-triol. One urine specimen obtained from a woman during the follicular phase of the menstrual cycle contained the pregnane-3,17,20-triol component only, while two specimens obtained from pregnant women contained the pregnane-3,20-diol component only. Neither component was present in the urine of an ovariectomized patient, although the colorimetric assay indicated that she had excreted 4.10 milligrams of "pregnanediol" in the 24-hour urine specimen.

Our data show that urine of normal women, in addition to pregnane-3,20-diol, usually contains a compound whose color reactions and Rf value are identical with pregnane-3,17,20-triol. During pregnancy, the urinary excretion of the latter compound tends to decrease.

## 9. SECONDARY HEMOCHROMATOSIS

MATTHEW BLOCK, PH.D., M.D., WILLIAM BETHARD, M.D. (BY INVITATION), AND LEON JACOBSON, M.D., CHICAGO, ILL.

One case of acute acquired hemolytic anemia and three cases of primary refractory anemia, two of which had hyperplastic erythroblastic marrows, have developed hemosiderosis, cirrhosis, auricular fibrillation, skin pigmentation, and decreased body hair. Diabetes developed in every case; only one patient had a familial history of diabetes. Three cases received massive and one ten transfusions. At autopsy the tissues were practically identical with those of patients with primary hemochromatosis.

Diabetes was mild, one case needing insulin; glycosuria was usually absent. Glucose tolerance of one case was decreased during ACTH therapy.

One patient had hemosiderosis of the marrow prior to therapy and marked hemosiderosis and slight scarring of the liver after only eight transfusions. At first iron was deposited primarily in hepatic epithelium at the periphery of the lobules, and scarring and bile duct proliferation were slight. By serial biopsy progressive increase in marrow and hepatic hemosiderosis, an accumulation of iron in all liver cells, and an increase in scarring and bile duct proliferation were demonstrable. In spite of intense hemosiderosis, parenchymatous cell degeneration was minimal, correlating with fairly normal liver function studies. Radioiron studies demonstrated an inability of one patient with primary refractory anemia to utilize radioiron; utilization was normal in the case of acquired hemolytic anemia.

These studies indicate that primary and secondary hemochromatosis are similar and possibly identical in regard to the type and progression of pig-

mentary cirrhosis, diabetes, skin pigmentation, loss of body hair, cardiac abnormalities, and terminal pathology. The major differences are the extreme chronic anemia present in patients with secondary hemochromatosis and the inability of patients with hemochromatosis secondary to primary refractory anemia to utilize iron for erythropoiesis.

These studies further indicate that patients with secondary hemochromatosis probably absorb abnormally large amounts of iron from the gastrointestinal tract early in their disease because marrow hemosiderosis precedes therapy and intense hepatic hemosiderosis occurs very early and possibly precedes therapy. This thesis is supported by the finding of others that the amount of iron in the tissue at autopsy far exceeds that obtainable from transfusions alone.

## 10. THE OXYGEN DIFFUSION CAPACITY OF THE ALVEOLAR-CAPILLARY MEMBRANE IN MITRAL STENOSIS

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(Introduced by Joseph H. Holmes, M.D.)

Pathologic alterations in the alveolar-capillary membrane have been described in patients with mitral stenosis. These findings suggested the possibility of an impediment to oxygen transfer at the alveolar-capillary membrane level in patients with mitral stenosis. Previous studies in this laboratory demonstrated an increased alveolar-arterial oxygen tension gradient in patients with mitral stenosis. The present study defines the components of this increased gradient.

Alveolar and arterial oxygen tensions were determined at two levels of oxygenation in ten patients with mitral stenosis. These values when employed in the formulas developed by Riley and associates permitted a calculation of the oxygen diffusion capacity of the alveolar-capillary membrane and the venous admixture fraction. Right heart catheterization was performed in all patients.

These studies demonstrated an impairment of oxygen diffusion in patients with mitral stenosis. This decreased diffusion capacity across the alveolar-capillary membrane accounted for a significant portion of the increased alveolar-arterial oxygen tension gradient in all patients.

Several patients demonstrated an increased percentage of venous admixture in addition to the diffusion impairment. The degree of impairment of diffusion was correlated with the pulmonary artery resistance, the pulmonary "arteriolar" resistance, and the pulmonary "capillary" pressure. This diffusion impairment represents a physiologic counterpart to the known pathological alteration in the alveolar-capillary membrane.

## 11. IN VITRO FUNGISTATIC ACTIVITY OF THE STILBENE DERIVATIVES—STILBAMIDINE, PROPAMIDINE, PENTAMIDINE, AND DIETHYLSTILBESTROL

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Elson, in 1945, demonstrated the fungistatic action of Propamidine, one of a group of aromatic diamidines used as trypanocides, against a number of pathogenic fungi, notably *Sporotrichum schenckii, Blastomyces dermatitidis, Achorion schoenleini,* and *Trichophyton sulfureum.* This was followed by re-

ports of encouraging results with the clinical use of Propamidine and stilbamidine on cases of cutaneous and systemic blastomycosis by Schoenbach and associates, Colbert and Curtis, and Harrell. In the light of these findings, experiments were performed using the Sabouraud agar dilution method to determine and compare the fungistatic spectra of the stilbene derivatives; stilbamidine, Propamidine, Pentamidine, and diethylstilbestrol, using twenty-two species of pathogenic fungi and three nonpathogens as test organisms. Because of promising results obtained in preliminary experiments with a number of steroid hormones, methyltestosterone was added. Isonicotinic acid was also included to determine if it showed fungistatic activity aside from its bacteriostatic effect against *Mycobacterium tuberculosis*. The several substances used changed the pH of the media not more than 0.2.

The most notable finding was the in vitro effectiveness of diethylstilbestrol against the strains of superficial and systemic fungi used, except *Phialophora verrucosa, Sporotrichum schenckii, Cryptococcus neoformans,* and *Candida albicans*. The sensitive strains included *Blastomyces dermatitidis, Blastomyces braziliensis, Histoplasma capsulatum,* and *Coccidioides immitis*. Complete inhibition of most strains was obtained with stilbamidine, Propamidine, and Pentamidine at a level of 1 mg. per milliliter of medium. Greater inhibitory power was exhibited by stilbamidine against *Hormodendrum pedrosoi* and *Blastomyces dermatitidis;* Pentamidine against *Hormodendrum pedrosoi* and *Sporotrichum schenckii;* and Propamidine against *Trichophyton ferrugineum, Cladosporium wernecki, Hormodendrum pedrosoi, Phialophora verrucosa, Sporotrichum schenckii, Blastomyces dermatitidis, Blastomyces braziliensis,* and *Coccidioides immitis*. Methyltestosterone showed certain fungistasis against a number of dermatophytes, *Blastomyces dermatitidis, Blastomyces braziliensis,* and *Histoplasma capsulatum*. Isonicotinic acid did not possess any fungistatic ability. Except for Alternaria which revealed some sensitivity to stilbamidine and Propamidine, the nonpathogens, Aspergillus and Penicillium, were resistant.

Clinical trials of these agents, particularly the stilbene, diethylstilbestrol, in the therapy of superficial and systemic mycoses are deemed worth while.

## 12. CAROTID PRESSURE STUDIES IN RELATION TO THE MANAGEMENT OF INTRACRANIAL ANEURYSMS

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(INTRODUCED BY A. C. CORCORAN, M.D.)

Ligation of the great vessels in the neck, particularly the carotid vessels, has been advocated as a practicable procedure in the management of bleeding intracranial aneurysms. This study concerns itself with changes in intra-arterial pressure following ligation of the common carotid artery as related to the management of these lesions.

Studies were carried out in ten cases of suspected intracranial aneurysms before and after common carotid artery ligation. Occlusion of the artery was accomplished by means of a modified Goldblatt clamp. Pressure recordings were made by the use of indwelling needles and catheters in the internal carotid artery at varying points in the neck and, in one case, intracranially at the time of craniotomy. Pressures were recorded by means of strain gauges, amplifiers, and a direct writing oscillography.

In the acute phase these studies showed an immediate significant reduction in intra-arterial pressure with an associated modification in the characteristics of the pressure curve. The additional occlusion of the external

carotid artery produced a further reduction in internal carotid artery intra-arterial pressure.

Chronic pressure studies on the same subjects, after maintained complete occlusion of the common carotid artery, showed a return of the intra-arterial pressure when measured at similar sites to near its original level within twenty-four and forty-eight hour intervals and probably sooner. The characteristics of the arterial pressure curve were similarly restored toward the pre-operative pattern.

The results of these studies tend to show that common carotid artery ligation is ineffectual in permanently reducing pressures distal to the point of ligation. The data in two cases suggested that combined ligation of the common carotid and the external carotid arteries may be somewhat more effective.

## 13. USE OF CITRATED HUMAN PLASMA FOR STUDYING THE NATURE OF BACTERIAL RESISTANCE TO STREPTOMYCIN

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Manometric studies of resting cells of *E. coli* indicate that streptomycin acts somewhere in the complex of enzymes required for bacterial metabolism of citrate. (Smith, Oginsky, and Umbreit: *J. Bact.* **58**: 761, 1949.) These methods employ simple substrates under conditions far removed from those encountered by streptomycin-resistant bacteria in vivo. In an attempt to study metabolism of citrate by streptomycin-resistant bacteria growing in an environment comparable to that encountered during human infection, we have inoculated streptomycin-sensitive and resistant strains of *A. aerogenes* into citrated human plasma.

Clotting of citrated human plasma by *A. aerogenes* has been shown to depend on consumption of citrate and is a simple end point for demonstrating citrate utilization. The mechanism of citrate utilization was studied by adding enzyme inhibitors. Addition of KCN prevented clotting by *A. aerogenes*, a fact which indicated that cytochromes or related iron enzymes were involved. Fluoroacetate, which interferes with oxidative metabolism of citrate in animals, also inhibited clotting by *A. aerogenes*.

Seven strains of *A. aerogenes* resistant to streptomycin in concentrations ranging from 8,000 to 50,000 $\mu$g per milliliter were developed from sensitive parent cultures originally isolated from human infections. The rate of clotting of citrated plasma by these resistant strains was consistently slower than that of sensitive parent strains. High concentrations of streptomycin, which did not restrict growth, repeatedly prevented utilization of citrate by all resistant strains of *A. aerogenes*. This was apparent from the following three effects of high concentrations of streptomycin: (a) permitted growth of resistant strains in citrated plasma but prevented clotting; (b) prevented resistant strains from destroying the anticoagulant action of citrated broth, although growth was unrestricted; (c) prevented clotting by newly developed resistant variants as they grew out in citrated plasma from initially sensitive populations.

It is concluded that streptomycin-resistant strains of *A. aerogenes* attack citrate more slowly than sensitive strains in the absence of streptomycin, and are diverted from the metabolic pathway involving citrate oxidation by high concentrations of streptomycin.

## 14. PULSE CURVES IN THE DIAGNOSIS OF CONGENITAL SUBAORTIC STENOSIS AND AORTIC STENOSIS

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The surgical treatment of valvular stenosis places renewed emphasis on the diagnosis of aortic stenosis and its differentiation from congenital subaortic stenosis. The latter occurs more frequently clinically than indicated by post-mortem incidence, and is relatively benign as an isolated lesion. Usually a fibrous ridge or bar just below but not involving the semilunar valves, congenital subaortic stenosis does not interfere with aortic valve mobility; whereas in aortic stenosis the valve is rigid.

Since the optically-recorded pulse curve from the subclavian artery retains all the characteristics of the aortic pulse; definite and graphic evidence of aortic valve mobility may be obtained. In a group of patients with suspected aortic valve obstruction, subclavian pulse tracings were recorded by means of a cup-tambour system using a Frank segment capsule and optical registration as described by Wiggers. Aortic and pulmonary area heart sounds and electrocardiogram were superimposed.

The normal subclavian pulse curve shows a rapid rise with a rounded peak falling to a sharp incisura with after vibrations associated with closure of the aortic valve. In aortic stenosis, the very gradual ascent of the pulse curve is broken by irregular vibrations, the graphic representation of the systolic murmur and thrill. On the catacrotic side the sharp incisura is lacking or rudimentary, and there are no after vibrations since valve rigidity prevents proper closure (and the second aortic sound is usually abolished). In subaortic stenosis, anacrotic vibrations and systolic murmur occur, but the diastolic fall is rapid and there is a sharp incisura followed by the normal after vibrations with no diminution of the second aortic sound.

Tracings considered characteristic of subaortic stenosis were obtained in ten patients, all of whom had the following in common: heart murmur in characteristic location present from infancy, normal physical and mental development, no symptoms referable to the lesion, and blood pressure within normal limits. Heart contour and electrocardiograms were usually within normal limits.

Subclavian pulse tracings give graphic evidence of aortic valve mobility and aid in distinguishing between aortic stenosis and congenital subaortic stenosis.

## 15. ANTIBODY TITER OF SERUM POOLS AS AN INDEX OF THE TYPES OF INFLUENZA VIRUS PRESENT IN A METROPOLITAN AREA

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Early recognition of the type of influenza virus causing an outbreak could make possible the prompt use of the appropriate type of vaccine and other protective measures. Present methods of detection of influenza involve virus isolation and observation of changes in the level of antibodies between acute and convalescent serum specimens. Both involve considerable delay before the type of virus responsible for the outbreak is determined.

The present study was undertaken to determine whether changes in the level of influenza antibodies in serum specimens collected from a representative

sample of the population would aid in detecting the prevalent type or types of influenza virus. Aliquots of serum were collected from patients admitted each week to five hospitals in the metropolitan area of St. Louis, Mo. These serum pools were examined for antihemagglutinins and complement-fixing antibodies against ten strains of influenza virus, six of which had been isolated in our laboratory.

At the same time acute and convalescent specimens of serum collected from cases of respiratory infection suspected of being influenza were examined for influenza antibodies. Virus isolations were attempted also but without success during the past year. During the study no epidemic of influenza occurred in St. Louis. However, in February, 1952, a moderate increase in respiratory infections was noted and a statement to this effect was made by the health commissioner.

Charts will be presented showing changes in titer of influenza antibodies week by week against the strains of virus studied. A well-marked increase in antibodies against type B virus was noted simultaneously with the rise in incidence of respiratory infections in February. Also in this period three cases were observed which showed a fourfold increase in titer of antibodies against type B virus. A less-marked increase in titer was noted against some strains of A prime virus and little or no increase against other A prime strains. Only slight activity against Type A was found and only a brief rise was observed against swine influenza virus. Low titers against all strains were found in the summer months of 1951 and 1952.

## 16. THE INFLUENCE OF DYNAMIC SEQUESTRATION OF AN ELECTROLYTE ON THE CALCULATION OF "SPACE" AND "MASS" BY THE TRACER METHOD

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During the course of continuous studies for 60 days with long-life isotopes, $Na^{22}$ and $Cl^{36}$, seemingly paradoxical phenomena in *calculated exchanging* sodium and chloride "space" and "mass" were observed. Some of these were: (a) increase in calculated space without gain in body weight; (b) decrease in calculated space with gain in weight; (c) increases in observed serum concentration of tracer without associated increase in concentration of nontracer; (d) discordant changes in *calculated* electrolyte mass and balance.

Data from measurement of tracer, chloride, sodium, and potassium in serum and urinary excretion support (a) anatomic and/or (b) functional isolation or sequestration of tracer at relatively high specific activity within various chloride compartments. Functional isolation can be produced by change in the state of chloride metabolism with "lags" in shifts of chloride among compartments. The data indicate spontaneous variations in size and state of chloride compartments occurring at intervals of hours to days with the apparent opening and closing ("blinking") of compartments resulting in erroneous calculations of *exchanging* or of total chloride space and mass. These observations, by the tracer technique, indicate further that: (a) calculations of exchanging space or mass of an electrolyte from a single determination can be grossly erroneous; (b) under certain circumstances estimation of *exchanging* space and mass can differ considerably from *actual* or total space and mass; (c) it is not possible to know when actual space and mass are obtained by calculation; (d) plot of successive measurements offers the best approach to actual space and mass because calculated values significantly different from mean ones are readily

detected; (e) anatomic areas involved in sequestration may be intracellular, osseous, and depots of poorly exchanging extracellular fluid such as ascitic, pleural, or spinal; (f) diuretics, changes in electrolyte intake, or variations in disease state may be factors responsible for changes in calculated space and mass; (g) there are obvious limitations of short-life isotopes for measurement of space and mass as well as many difficulties concerned with space measurements by any tracer, isotopic or nonisotopic; (h) previously seemingly paradoxic data are now interpretable.

## 17. IMMUNOLOGIC STUDIES OF FETAL HEMOGLOBIN

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(Introduced by Carl V. Moore, M.D.)

Techniques have been developed, using anti-fetal hemoglobin serum of high potency and specificity, for the qualitative and quantitative determination of fetal hemoglobin (F hgb.) in human blood specimens. The production of anti-hemoglobin sera of high titers has been made possible by the use of adjuvant mixtures to increase the antibody response evoked by the weakly antigenic material. In the present investigation, pure fetal hemoglobin has been employed as antigen. The latter was separated from other forms of human hemoglobin by taking advantage of the resistance of fetal hemoglobin to denaturation by highly alkaline reagents. Absorption studies employing the normal adult, sickle-cell, and fetal varieties of hemoglobin suggest that the anti-fetal serum is specific for the embryonic pigment. A quantitative precipitin reaction, based upon a straight line relationship between the logarithm of the time in minutes required for the precipitin ring to become apparent and the logarithm of the amount of F hgb. has permitted the estimation of extremely small quantities of this compound, reproducible results being obtained with as little as 0.03 per cent F hgb.

Results of the study reveal that: (a) fetal hemoglobin occurs in normal adult individuals in small amounts, the maximum being less than 1.0 per cent of the total hemoglobin, and, in general, less than 0.5 per cent. Approximately 30 per cent of a series of 150 normal adults had less than 0.03 per cent F hgb. (b) The alkali-resistant hemoglobin fractions detected the denaturation technique in many instances of the hereditary hemolytic syndromes and occasionally in acquired hematologic diseases are identical, immunologically, with normal fetal hemoglobin. Comparative results between the values for F hgb. obtained by the alkali denaturation technique and the quantitative precipitin test were in excellent agreement in the range between 2.0 per cent and 10.0 per cent. The error in reading the precipitin test above 10.0 per cent F hgb. became progressively greater, while the denaturation technique is insensitive to amounts of less than 2.0 per cent of this compound. (c) Although abnormal quantities of F hgb. are almost always found in the hereditary hemolytic anemias, no specific pathogenetic significance could be ascribed to this observation.

## 18. OBSERVATIONS ON METABOLIC EFFECTS OF REPLACEMENT THERAPY (CORTISONE, DOCA, AND TESTOSTERONE) IN PATIENTS WITH ADDISON'S DISEASE

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A study was conducted on four adult male patients with Addison's disease. Fifty milligrams of cortisone daily with a 6 Gm. sodium diet could maintain a

positive sodium balance. Sodium balance was more positive with 50 mg. of cortisone and 2.5 mg. of DOCA than 25 mg. of cortisone and 2.5 mg. of DOCA. Nitrogen balance may become negative on 50 mg. of cortisone alone. Testosterone in dosage used showed no increase in positive nitrogen balance. Potassium balance with 3 Gm. intake showed no great alteration on any of the regimes. Intravenous glucose tolerance test was not significantly influenced by 50 mg. of cortisone given up to six weeks, curves remaining flat. Urinary neutral 17-ketosteroids and corticoids showed a significant increase on 50 mg. of cortisone. Cortisone induced an initial eosinopenia which was not always sustained. With DOCA a definite rise in eosinophils was obtained in some patients. Kepler "A" values were restored to normal by cortisone and DOCA, while 24-hour endogenous creatinine clearance increased but remained below accepted normal range per square meter body surface. Blood pressure could be raised only to lower normal limits. Addisonian patients with bronchial asthma and chronic urticaria became free of symptoms.

## 19. A METABOLIC EVALUATION OF THE RABEN-WESTERMEYER GROWTH HORMONE IN A PITUITARY DWARF WITH COEXISTING DIABETES MELLITUS

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Purified growth hormone, prepared from beef-pituitary glands by either the Wilhelmi or the Li method, although clearly growth promoting in animals, has failed to produce positive nitrogen balance in man. Another perplexity, as well as a potential clinical hazard, is the fact that such preparations, while growth promoting in puppies, produce permanent diabetes mellitus in adult dogs. This relationship between growth hormone activity and pancreatic islet cell function has given rise to a number of hypotheses regarding the role of insulin in the growth process as well as the influence of the growth hormone in the etiology of diabetes mellitus.

Raben and associates have reported recently upon the isolation of a hog pituitary fraction, which is growth promoting in animals, is not diabetogenic in adult dogs, and which produced nitrogen retention without effecting carbohydrate metabolism in a woman with hypopituitarism. Since the experiment in man was of short duration, it did not eliminate potential diabetogenicity if used clinically for growth promotion over a prolonged period.

In the Houssay animal small amounts of diabetogenic pituitary preparations produce a rapid and severe recrudescence of the diabetic state. The subject of our study is a 17-year-old boy with panhypopituitarism due to a calcified craniopharyngioma plus the very rare coexistence of diabetes mellitus. Thus, he presents a metabolic state which approaches that of the Houssay preparation.

The results of a detailed metabolic balance study over a 24-day period indicate the following: (a) significant anabolism of body protein was produced in this human being with the Raben-Westermeyer growth preparation; (b) no intensification of the diabetic state was observed; (c) the anabolic effect of growth hormone was not intensified by administration of insulin; (d) insulin alone was definitely protein anabolic in this diabetic pituitary dwarf, who without insulin maintained nitrogen equilibrium.

It is believed that the Raben-Westermeyer preparation when given intramuscularly below pH4 can be expected to induce growth in human pituitary

dwarfism without the risk of inducing diabetes mellitus. This study, however, raises for the investigator a number of new questions which are not answered easily on the basis of present concept.

## 20. BLOOD FLOW AND OXYGEN CONSUMPTION OF THE BRAIN IN COARCTATION OF THE AORTA

CHARLES W. CRUMPTON, M.D. (BY INVITATION), ROBERT T. CAPPS, PH.D. (BY INVITATION), JOHN H. MOYER, M.D., AND JOSEPH H. HAFKENSCHIEL, M.D. (BY INVITATION), MADISON, WIS.

Reports concerning the circulatory alterations in coarctation of the aorta have not established decisively whether or not vascular resistance is increased uniformly throughout the body. Studies in our laboratory have indicated that there is increased resistance to blood flow through the brain in essential hypertension; this region sharing in the generalized increase in vascular resistance which is associated with the disorder. We propose to present evidence that the cerebral hemodynamics in eight patients with coarctation differ from those with essential hypertension.

Quantitative determination of cerebral blood flow by means of the nitrous oxide method was performed in eight patients with coarctation of the aorta. The cerebral blood flow, cerebral oxygen consumption, and mean brachial arterial pressure were significantly increased ($p < 0.01$) as compared with Kety's mean values obtained in thirty-four studies on fourteen normal young men of a comparable age group. The cerebral vascular resistance was normal in coarctation, whereas it was increased in seventy patients with essential hypertension. Postoperatively, four patients with coarctation showed a decrease in cerebral blood flow (68 to 53 c.c. per 100 Gm. per minute), cerebral oxygen consumption (4.2 to 3.3 c.c. per 100 Gm. per minute) and mean brachial arterial pressure (114 to 95 mm. Hg). Cerebral vascular resistance was unchanged.

Data obtained from study of the cerebral hemodynamics in eight patients with coarctation of the aorta indicate that cerebral vascular resistance is within the normal range. These observations tend to support the thesis that there is no generalized increase in vascular resistance in coarctation.

## 21. EFFECT OF CYSTEINE ON THE IMMUNE RESPONSE AND MORTALITY RATE OF RABBITS EXPOSED TO IONIZING RADIATION

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This study is concerned with the relationship of the immune response to survival following exposure of rabbits to whole body irradiation. Foreign proteins administered before exposure to ionizing radiation (IR) reduce the mortality rate and also protect the immune response. It was reasoned that if those agents which protect the immune response also reduced the mortality rate, a survey for agents capable of increasing the survival time and rate, particularly in studying their action in larger experimental animals, would be immeasurably simplified. Since cysteine had been studied extensively (Patt, Tyree, Straube, and Smith) and shown effective in improving survival of rats when administered before exposure to IR, it was investigated more extensively than other agents used in this study.

The immune response was measured by determining the rate of antigen ($I^{131}$-labeled bovine gamma globulin [Armour]) elimination from the blood.

The sensitivity of the method was increased by administering the antigen six hours after wholebody exposure of the rabbit to an LD 50/30 dose (800 r). Cysteine HCl (0.5 Gm. per kilogram) was given five minutes before exposure. It was neutralized rapidly with NaOH within the syringe immediately before intravenous injection, to reduce oxidation. Control rabbits were given 5 ml. of 5 per cent NaCl intravenously.

In the control rabbits, the average blood antigen level on the fourth day was 11 per cent of the injected amount, the seventh day, 5 per cent, the tenth day, 1.8 per cent, and the fourteenth day, in the range of 0 to 0.3 per cent. The blood antigen levels of the cysteine-injected rabbits closely approximated those of the controls, indicating that the immune responses of the two groups were comparable. The numbers of rabbits in each group surviving on the tenth, twentieth, and thirtieth days also were comparable. With 500 r and cysteine being given to one experimental group before, and to the other group after exposure, rates of antigen elimination were comparable to those of the control rabbits.

Thus, under the conditions described, cysteine administered intravenously to rabbits before whole-body exposure to an LD 50/30 dose (800 r) of ionizing radiation did not modify either the immune response or the survival rate.

## 22. THE ROLE OF MATURATION ARREST IN THE LOW ERYTHROCYTE COUNTS OF PERNICIOUS ANEMIA

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Fresh normal erythrocytes belonging to group O were transfused to six patients with pernicious anemia in relapse belonging to group A or B. Donors' cells were identified in the circulation of the patients by Ashby's method of differential agglutination. Analysis of inagglutinable erythrocyte counts demonstrated that the normal donors' erythrocytes were so affected by twenty-four hours or more in the contact with a deficiency of $B_{12}$ in the body of the patients that the transfused cells were randomly destroyed at rates that could be measured rather accurately.

At the beginning of the transfusion experiments the erythrocyte levels of the patients were in steady states with counts of 1.0, 1.64, 1.70, 2.00, 2.00, and 3.5 millions per cubic millimeter. In each the aspirated bone marrow presented the cytologic picture which has been considered typical of "maturation arrest." The subsequent administration of $B_{12}$ parenterally resulted in prompt rises of the patients' erythrocyte counts to normal and disappearance of all symptoms and signs of the disease except those attributed to permanent changes in the spinal cord.

Assuming a normal rate of erythropoiesis when the cells are being destroyed by a hemolytic mechanism, the resulting lowered level of the erythrocyte count can be calculated, if the rate of destruction is known. In these studies it was assumed that the rates of destruction of the patients' erythrocytes were similar to the measured rates at which the biologically tagged cells disappeared.

In four patients the observed levels of erythrocyte counts before treatment coincided with those calculated on the assumption of normal erythropoietic rates and the observed random loss of donors' cells. The lowered cell counts could therefore be explained completely by the action of a hemolytic mechanism and without maturation arrest of significant magnitude. In the other two patients this method of calculation suggested that about one-half of the lowering of the

erythrocyte count could be attributed to a hemolytic mechanism, whereas the other one-half was explainable by a phenomenon such as maturation arrest. Reticulocyte response to $B_{12}$ was significantly lower than expected in those in which the hemolytic mechanism predominated.

## 23. A STUDY OF THE NATURE OF THE CIRCULATING THYROID HORMONE IN EUTHYROID AND HYPERTHYROID SUBJECTS BY USE OF PAPER ELECTROPHORESIS

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(INTRODUCED BY OVID O. MEYER, M.D.)

It is generally accepted that the circulating thyroid hormone is the amino-acid thyroxine loosely bound to a plasma protein. Using paper electrophoresis to separate the plasma protein fractions and $I^{131}$ as a tracer isotope, it is possible to study the nature and behavior of circulating iodine-containing compounds. The pattern derived from this combination of techniques has been called a radioelectrophoretogram.

The radioelectrophoretograms of euthyroid and hyperthyroid subjects were studied at intervals for several weeks following the administration of a single dose of carrier-free $I^{131}$. After appropriate corrections were made for isotope decay and differences in dosage, the patterns of these two groups of patients were compared. Initially, most of the $I^{131}$ was unbound by protein, although there was a definite and progressive localization in the albumin fraction. Within three days virtually all of the radioactivity was protein bound. In the normal subjects, in addition to the albumin-bound radioactivity, there appeared after several days an area of radioactivity just ahead of the alpha 2 area. The radioactivity at this site gradually increased. The same sequence of localization appeared in the hyperthyroid subjects. However, the radioactivity in the alpha globulin area appeared sooner, increased more rapidly to much higher levels, and then declined sharply.

$I^{131}$ added as sodium iodide to serum becomes partially albumin bound. Radioactive thyroxine added to serum is bound to a protein in the alpha globulin section. It appears then most likely that the circulating hormonal iodine is carried by this alpha-globulin fraction.

## 24. SERUM PROFIBRINOLYSIN AND ANTITRYPSIN TITERS AND IN VITRO LYSIS OF WHOLE BLOOD CLOTS OF IRRADIATED RABBITS

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Fibrinolytic changes have been observed in the hemorrhagic diathesis of irradiated goats, pigs (Cronkite), and dogs (Jackson and associates, Colgan and associates). This study was undertaken to assess the fibrinolytic changes in the postirradiation syndrome in rabbits.

Ten rabbits, weighing from 3.5 to 4.5 pounds, were given 900 r total body irradiation each. Determinations of (a) serum profibrinolysin titers, (b) serum antitrypsin titers (Christensen's method), (c) clot lysis, (d) red blood cell, and (e) platelet counts were done before, and every other day after irradiation. The rabbits succumbed before the eighth postirradiation day. Two rabbits, with thrombocytopenia and anemia, died with traumatic hemopericardium shortly after cardiac puncture on the sixth and seventh postirradiation days, respectively.

The other rabbits showed no thrombocytopenia, nor gross hemorrhages at autopsy. Profibrinolysin titers (ranging from 1/40 to 1/120 before irradiation) increased after irradiation, the final titers ranging from 1/60 to 1/200. The antitrypsin titers (ranging from 1/20 to 1/30 before irradiation) increased after irradiation to a final titer range of 1/30 to 1/50. These increases exceed those one would expect from diminution in plasma volume alone (Storey and associates). No rapid clot lysis was observed. Thus in irradiated rabbits elevations in serum profibrinolysin and anti-trypsin titers may take place in the absence of spontaneous hemorrhages.

## 25. PHENYLINDANDIONE, A NEW ORAL ANTICOAGULANT

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The hypoprothrombinemic effect of phenylindandione (PID) has been compared with Dicumarol and Tromexan in patients with thromboembolic disease. Anticoagulant dosage was based on daily determination of "prothrombin concentration" (Quick method) and the therapeutic goal was reduction to less than 30 per cent. An average initial PID dose was 400 mg., followed in the next twenty-four hours by 300 to 400 mg. in two doses. The average subsequent daily maintenance figure was 130 milligrams. A divided schedule achieved the most stable hypothrombinemic effect. Comparison of the anticoagulant effect of PID with two popular coumarin derivatives was made as shown in Table I.

TABLE I

| | PID | DICUMAROL | TROMEXAN |
|---|---|---|---|
| Patients treated | 65 | 122 | 50 |
| Mean effective dose | 700 mg. | 646 mg. | 3120 mg. |
| Achievement of therapeutic effect within 36 hours | 66 per cent | 32 per cent | 22 per cent |
| Average (in days) | 1.4 | 2.25 | 2.1 |
| Average days to recover to 40 per cent or above | 1.6 | 5.0 | 2.5 |
| Incidence of prothrombin: | | | |
| "escape" above 30 per cent | 18 per cent | 37 per cent | 90 per cent |
| fall below 10 per cent | 14 per cent | 15 per cent | 20 per cent |

The therapeutic level of prothrombin concentration was achieved most quickly with PID. The mean effective initial dose of PID was comparable to Dicumarol, but the recovery period, however, was shorter. With appropriate divided doses of PID a consistent effect on prothrombin activity was achieved for prolonged periods. The development of bleeding (5 per cent in 100 patients) emphasizes that PID shares this hazard, common to all anticoagulants. Vitamin $K_1$ proved an effective antagonist to PID.

## 26. TRANSMISSION OF ELECTROCARDIOGRAM OVER STANDARD TELEPHONE LINES

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(INTRODUCED BY WILLIAM C. MENNINGER, M.D.)

If the electrical energy arising in the heart which produces the electrocardiogram could be transmitted over a standard telephone wire and, at the re-

ceiving end, the electrocardiogram could be reproduced, an extremely useful technique could be developed. In military medicine, a central general hospital with competent cardiologists could give immediate consultation to outlying units. Similarly, in civilian life, practitioners in rural areas could contact a distant consultant by telephone, give him the entire history, and then an immediate answer as to the significance of the patient's electrocardiogram.

We have developed such a transmitter and receiver and have satisfactorily transmitted the electrocardiogram over long-distance telephone lines. Our equipment employs a standard electrocardiograph machine as part of the transmitting equipment. The voltage existing across the galvanometer coil of the standard electrocardiograph machine is used to frequency modulate a 1,500 cycle tone which can be transmitted over the telephone line. At the receiving end, this frequency modulated tone or carrier is converted back to the original modulating frequency and a duplicate of the original electrocardiogram recorded.

## 27. SERIAL SERUM CHOLESTEROL IN CHILDREN WITH DIABETES MELLITUS OF RECENT ONSET

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Altered lipid metabolism and altered carbohydrate metabolism are being emphasized in the study of diabetic patients. Many juvenile diabetic patients who have had their disease over ten years are known to be developing vascular disease. Interest in the cholesterol values of children with diabetes is a natural consequence of the recognition that a relationship exists between altered lipid metabolism and atherosclerosis, and these patients are in an age group not expected to develop significant vascular degenerative changes.

A total of four hundred thirty-five serial serum cholesterol values were determined in forty-eight children with diabetes mellitus early in the course of their disease.

Before insulin therapy was instituted a larger number of elevated values were observed, but there were many values within the normal range. Marked fluctuations in cholesterol values in individual patients with diabetes mellitus were noted, particularly in the early weeks of treatment. Hydration accounted for a decrease in cholesterol values in five patients. The average cholesterol values increased in the first three weeks of therapy and then gradually decreased to a value below the average for normal children. During the period of home management (maximum two years) the patients who maintained an excellent or good level of control tended to maintain cholesterol values within the normal range. Those children who maintained only a fair to poor level of control during the period of home management continued to show marked fluctuations in cholesterol values.

Cholesterol values showed a significant decrease in five patients with mild or severe infection. No correlation was found between the cholesterol values, the insulin dose, the age of the patient, or the sex of the patient.

The changes, or lack of changes, in cholesterol values early in the course of treatment are important in evaluating changes occurring later. This study serves as a base for evaluating the usefulness of various types of therapy and the importance of level of control as a factor in the maintenance of normal lipid, as well as normal carbohydrate metabolism, and their relation to the prevention of degenerative disease in juvenile diabetics.

## 28. THE EFFECT OF STRESS ON GLUCOSE AND FRUCTOSE METABOLISM

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The mechanism by which stress influences carbohydrate metabolism has not been completely elucidated. According to Selye's concepts, stress causes an excessive production of adrenocortical (ACH) and anterior pituitary hormones (APH). It has been advanced by Cori that insulin acts to protect the phosphorylation of glucose by blocking the inhibitory action of these hormones. In the stress state it would be reasonable to expect that due to an excessive production of APH and ACH, insulin action is relatively less effective. One should, therefore, find a decreased tolerance for glucose, but the tolerance for fructose, whose phosphorylation is independent of insulin action, should not be affected by stress.

To test this hypothesis, the stresses of an acute febrile illness and surgery were studied relative to glucose and fructose tolerance. Five patients were given intravenous glucose and fructose tolerance tests on separate occasions before and after surgery. Thirty-five patients with typhoid fever were studied during the acute illness and convalescence, thirty-four for glucose tolerance, and one for tolerance to both glucose and fructose.

The results of these studies indicate the tolerance for glucose as estimated by the blood sugar curve, hexose excretion, changes in pyruvic acid, and phosphorus is diminished by the stress of surgery or an acute febrile illness (typhoid). The tolerance for fructose, however, was unaltered in the surgical patients and in the typhoid case studied. These results will be discussed with regard to intravenous alimentation and the nature of the physiologic derangement of carbohydrate metabolism induced by stress.

## 29. SUPERSENSITIVITY OF THE BLOOD VESSELS OF HUMAN SKELETAL MUSCLE TO ADRENALINE FOLLOWING SYMPATHECTOMY

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(INTRODUCED BY JAMES W. CULBERTSON, M.D.)

The occurrence in man of supersensitivity of the skin vessels to the constrictor effect of Adrenalin following sympathectomy was described by Freeman, Smithwick, and White (*Am. J. Physiol.* **107**: 529, 1934). Postganglionic denervation was thought by White, Okelberry, and Whitelaw (*Arch. Neurol. Psychiat.* **36**: 1251, 1936) to cause much greater supersensitivity than preganglionic decentralization, in accordance with Cannon's law of denervation. Fatheree, Adson, and Allen (*Surgery* **7**: 75, 1940) stated, on the contrary, that both operations produce an equal degree of sensitization of the skin vessels. The present study is an attempt (1) to discover if surgical sympathectomy causes supersensitivity of the vessels in skeletal muscle, and (2) to determine whether postganglionic denervation causes a greater degree of supersensitivity than preganglionic section.

The blood flow responses in muscular segments of the upper and lower limbs of 35 healthy subjects and 15 sympathectomized patients were measured

plethysmographically during intravenous and intra-arterial infusions of Adrenalin. In normally innervated subjects the steady intravenous infusion of 10 $\mu$g of Adrenalin per minute causes a biphasic increase in muscle blood flow, an initial brief but marked vasodilatation being replaced by a blood flow rate slightly above the control level (Allen, Barcroft, and Edholm: *J. Physiol.* **105**: 255, 1946). In sympathectomized limbs the initial dilator phase was found to be greater than normal and the subsequent phase of moderate dilatation was replaced by relative or absolute vasoconstriction.

The findings were thought to indicate supersensitivity of sympathectomized blood vessels in skeletal muscle to both phases of the direct action of Adrenalin. The degree of supersensitivity in a group of lower limbs sympathectomized by preganglionic decentralization was found to be no less than that found in a group of upper limbs sympathectomized by either preganglionic section or ganglionectomy. The study thus presents evidence in conflict with Cannon's law of denervation.

## 30. BIOPSY OF THE LUNG AND CARDIAC CATHETERIZATION STUDIES IN PATIENTS TREATED SURGICALLY FOR MITRAL STENOSIS

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To study the functional significance of structural changes in the pulmonary vasculature occurring in the presence of mitral stenosis, specimens of pulmonary tissue were obtained in 25 cases at the time of mitral commissurotomy. The degree of the structural vascular changes was compared with resistance to pulmonary flow, total pulmonary flow, and pulmonary arterial pressures as determined by cardiac catheterization before and after commissurotomy. An attempt also was made to determine whether there was a correlation between the degree of these changes and electrocardiographic evidence of right ventricular hypertrophy.

Preoperatively, the pulmonary arterial pressures were elevated above the range of normal in all 25 cases; by cardiac catheterization the average systolic pressure was found to be 76 mm. Hg (range: 126 to 45), and the average diastolic pressure, 40 mm. Hg (range: 64 to 24). Postoperative catheterization studies were done in 10 cases. The average systolic pressure was 57 mm. Hg (range: 80 to 32), and the average diastolic pressure was 28 mm. Hg (range: 45 to 17). Structural alterations in the pulmonary vessels were observed in every case. The most consistent change was pronounced medial hypertrophy of the large muscular arteries and moderate medial hypertrophy of the small muscular arteries. Intimal fibrous thickening of the arteries and of the arterioles was present in varying degrees. The pulmonary veins showed alterations in every case.

A favorable postoperative response, as measured by cardiac catheterization, consisted of a significant reduction in the pulmonary arterial pressure, the pulmonary capillary pressure, the total pulmonary resistance, and the pulmonary "arteriolar" resistance.

The degree of the structural vascular changes in patients with the most favorable postoperative responses, as measured by physiologic studies, was frequently similar to that encountered in patients with less favorable postoperative responses. Thus, the pathologic changes in the pulmonary vessels could not be used as a reliable index for predicting whether or not a satisfactory

functional response could result from commissurotomy. A better basis for prediction regarding functional improvement could be obtained from the surgeon's appraisal of the valve at the completion of mitral commissurotomy.

## 31. STEROID COMPOUNDS RESULTING FROM INCUBATION OF CORTISONE WITH SURVIVING LIVER SLICES

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(INTRODUCED BY ROBERT E. SHANK, M.D.)

The ability of surviving liver tissue to alter the chemical structure of various steroid hormones has previously been demonstrated. These studies have been concerned primarily with inactivation of androgenic and estrogenic substances. Adrenal steroids have not been extensively studied in this manner, although Schneider has recently reported catabolism of cortisone by rapid reduction of the conjugated unsaturated bonds in ring A of the molecule and extensive degradation of the alpha-ketol side chain when incubated with surviving rat liver slices.

The purpose of the present study was to determine if cortisone is actively metabolized by surviving liver slices, and to detect and characterize any new compounds that might result from such metabolic processes.

Rat liver slices were incubated aerobically in phospho-saline buffer in the presence of small amounts of cortisone. After incubation for three hours at 37° C., the steroid fraction was recovered by extraction and analyzed by paper chromatography. Chromatograms demonstrated the presence of three steroid compounds.

The steroids were eluted and then characterized by determination of absorption spectra in ultraviolet light, by formation of sulfuric acid chromogens, by certain color reactions, and by comparison of mobility on paper with that of known compounds.

The compound recovered in greatest quantity and which moved most rapidly on paper was cortisone, unchanged by incubation. The second compound, obtained in least amount and of intermediate polarity, proved to be Compound F. The third and most polar steroid, obtained in somewhat greater quantity than Compound F, is unidentified. Analysis indicates that this substance retains the conjugated unsaturated bonds in ring A, but the alpha-ketol side chain at C-17 is altered.

The results demonstrate that the liver can convert cortisone to Compound F. Since Compound F is biologically active, this evidence indicates that the liver metabolizes steroids not only by inactivation but may produce such alterations in the molecule that other active hormonal substances are formed.

## 32. CHRONIC HYPEROSMOLARITY OF THE BODY FLUIDS

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(INTRODUCED BY JOHN S. HIRSCHBOECK, M.D.)

Hypertonicity of the body fluids has been described in association with different lesions of the central nervous system in different locations. The cause of the electrolyte disturbance may not be entirely identical in these different cases. However, the most common cause of hypertonicity of the body fluids is dehydration. This situation normally is safeguarded by the subjective sensation of thirst.

Studies were made on a patient with a cerebral lesion which was responsible for the development of diabetes insipidus. She exhibited chronic hyperchloremia (110-120 meq. of chloride per liter) and hypernatremia (150-165 meq. of sodium per liter), a defective thirst mechanism and, in addition, definite evidences of anterior pituitary insufficiency. Two patients with uncomplicated diabetes insipidus were studied for comparison. The patients were studied before, during, and after the administration of 0.3 ml. of Parke-Davis Pitressin intramuscularly every four hours for three days. So as to evaluate thirst mechanisms, all were allowed fluids ad libitum. Cortisone (25 mg. per day) was administered to the patient with hypernatremia throughout one study period; it was omitted during another.

The results were as follows: the patient with chronic hypernatremia ingested relatively constant amounts of water throughout, even though the hypertonicity of the body fluids was alleviated by Pitressin. After discontinuance of Pitressin the hyperosmolar state promptly recurred mainly through a process of salt retention when receiving cortisone and through water diuresis when not. Pitressin did not cause increased renal losses of salt. In another study the administration of 100 mg. of cortisone daily for four days did not aggravate the diabetes insipidus. Thus, no direct antagonism between the antidiuretic hormone and cortisone on the renal tubular absorption of water or salt was demonstrated. Prolonged administration of Pitressin Tannate in oil resulted in hyponatremia and edema in a later study.

Salt retention rather than diuresis of water characterized the first 48 hours after Pitressin in the patients with uncomplicated diabetes insipidus. It is suggested that adrenal cortical activity, but not necessarily increased activity, permits this reaction.

Some of the reported cases of hypernatremia in patients with neurologic lesions may represent examples of diabetes insipidus and a disturbed thirst mechanism.

## 33. STUDIES ON THE METABOLISM OF COMPOUND F (17-HYDROXY-CORTICOSTERONE)

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(INTRODUCED BY JOHN S. HIRSCHBOECK, M.D.)

It is recognized that 17-hydroxy-corticosterone (Kendall's Compound F) has as great or greater biologic activity as Compound E (cortisone). Studies by others have indicated that Compound F is at least the principal so-called "glycogenic" adrenal cortical steroid. However, Conn and associates (*Science* **113**: 713, 1951) postulated that because of its widespread actions it is the only steroid discharged by the normal adrenal. Because orally administered free Compound F or its acetate caused increased amounts of the neutral 17-ketosteroids to appear in urine, it was further suggested that it was unnecessary to consider cortical elaboration of separate "androgenic" steroid(s) as a source of these urinary products.

If compound F is the only steroid discharged by the adrenal, its administration to individuals with adrenal cortical insufficiency should provide complete and entirely physiologic replacement therapy. To test this, a 60-year-old woman with adrenal cortical insufficiency secondary to anterior pituitary insufficiency was given Compound F acetate, orally, on two separate occasions, in amounts sufficient to bring the low levels of urinary 17-ketosteroids to within the normal range. It was administered by the unphysiologic oral route so that maximal transformation to urinary steroid would occur. From these and

other data it appeared that approximately 200 mg. of the steroid per day was necessary to bring the level of the urinary 17-ketosteroids to normal. At this dosage the serum bicarbonate rose to abnormal levels and the potassium and chloride of the serum fell. That a new but abnormal biologic equilibrium (hypochloremic alkalosis) had been induced was substantiated by the striking retention of potassium and chloride that occurred after discontinuance of the hormone. Thus, while it is possible that not all individuals with adrenal cortical insufficiency will develop the observed electrolyte disturbance at this dosage, the data lend support to the idea that Compound F is not the only steroid elaborated by the normal adrenal, and that other precursors of the urinary 17-ketosteroids may also be formed.

## 34. THE EFFECT OF A HEAD-UP TILTED POSITION ON RENAL HEMODYNAMICS AND WATER AND ELECTROLYTE EXCRETION IN PATIENTS WITH HYPERTENSION AND RENAL DAMAGE

RALPH V. FORD, M.D. (BY INVITATION), LEWIS C. MILLS, M.D. (BY INVITATION), AND JOHN H. MOYER, M.D., HOUSTON, TEXAS

Renal function was determined by standard techniques in twenty male patients with hypertension. There was associated renal damage in fourteen cases as indicated by GFR less than 95 ml. per minute (inulin and creatinine clearance). Glomerular filtration rate, renal plasma flow (PAH clearance), TM PAH, and the rate of water, sodium, and potassium excretion were determined in the control state and after 60 degrees head-up tilting.

The patients were arbitrarily divided into four equal groups on the basis of control RPF in ascending order. There was no significant difference in the response between any of these four groups of patients or in a group of patients with normal blood pressure. There was a 10 per cent drop in mean blood pressure in the tilted position, but a 29 per cent drop in GFR and 28 per cent drop in RPF indicating an increase in renal vascular resistance. There was also an 18 per cent drop in TM PAH, indicating a decreased number of functioning nephrons as well as a decrease in filtration in those nephrons remaining. The hematocrit was not significantly altered (5 per cent increase). The rates of water and sodium excretion were depressed (50 per cent and 60 per cent, respectively) more than any other process studied. Potassium excretion was depressed 25 per cent.

The data indicate that the tilted position inaugurates a series of vasoconstrictive influences at the expense of normal renal hemodynamics, and also results in a decline in water and sodium excretion greater than the increase in renal vascular resistance. The data show no difference in reactivity to these vasoconstrictive influences in the kidney of a patient with hypertension as compared to normal subjects. The presence of renal damage did not alter this response.

## 35. METABOLIC AND RENAL EFFECTS OF PROBENECID (BENEMID)

RALPH V. FORD, M.D. (BY INVITATION), AND C. L. SPURR, M.D., HOUSTON, TEXAS

In an attempt to correlate and further elucidate the mechanisms of action of probenecid, five patients with gout have been observed under metabolically controlled conditions. The patients were studied in three groups of periods: pretreatment control, probenecid treatment, and post-treatment control. Diet,

feces, and urine were analyzed by standard methods for the determination of nitrogen, phosphorus, sodium, potassium, and uric acid balances. Standard differential renal function tests were performed by standard techniques. Glomerular filtration rate (inulin and creatinine clearances), renal plasma flow (PAHA clearance), and maximum tubular excretory capacity for PAHA (TM PAH) were determined in addition to uric acid clearances.

The data indicate no effect on nitrogen, sodium, and potassium balance. The urinary excretion of phosphorus was not altered, but there was an apparent increase in fecal phosphorus excretion (to an average of 287 per cent of control) due to the drug. The renal functions showed no change in GFR or RPF, but there was a depression of TM PAH to 66 per cent of control (or from 83 to 59 mg. per minute) due to the drug. The uric acid clearance was increased to an average of 210 per cent of control, tubular reabsorption of uric acid was depressed to 45 per cent of control, and the ratio of uric acid clearance to inulin clearance was increased to 198 per cent of control. All these effects returned to the control values in the post-treatment periods.

These observations suggest a selective reversible inhibition of uric acid reabsorption and PAHA excretion by the renal tubules without change in filtration rate. This is in agreement with previous observations in dogs and men. However, our observations on phosphorus balance seem to demonstrate an additional action of probenecid, indicating a reversible inhibition of intestinal absorption of phosphorus in the absence of an effect on the renal tubular reabsorptive mechanisms of this anion.

## 36. BILIARY CIRRHOSIS WITH CUTANEOUS XANTHOMATOSIS DUE TO SARCOIDOSIS: TREATMENT WITH LOW CHOLESTEROL DIET, ACTH, AND CORTISONE

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A Negro man, aged 24 years, with a severe chronic febrile illness of two years' duration, developed jaundice, hepatosplenomegaly, extreme elevation of the serum lipids, and extensive cutaneous xanthomatosis. The serum remained transparent. Lymph node and liver biopsies showed granulomatous lesions typical of Boeck's sarcoid. Prolonged treatment with penicillin, dihydrostreptomycin, para-aminosalicylic acid, and aureomycin did not alter the clinical course. On a low cholesterol, low fat diet the total serum cholesterol fell from 1,875 mg. per cent (6 per cent esters) to 254 mg. per cent (37 per cent esters) in a period of less than six months. Correspondingly, the serum bilirubin fell from 10.6 mg. per cent to 7.1 mg. per cent, and the alkaline phosphatase from 67.7 B.U. to 55.3 B.U. Administration of ACTH and later cortisone was associated with a prompt further increase in the per cent of cholesterol esters (up to 75 per cent), while the total cholesterol remained within normal limits. The high serum globulin returned to normal. In addition, hormone therapy suppressed the fever, reduced the sedimentation rate, and exerted a beneficial clinical effect on appetite and mental outlook. The xanthomata disappeared, and there has been an appreciable decrease in the hepatosplenomegaly. The patient, although still icteric, has done well on oral cortisone (50 mg. daily) for the past year and one-half. He has been able to resume moderate activity at home for the past year. The 17-ketosteroid excretion has remained unchanged (12 to 15 mg. per 24 hours) while on cortisone.

## 37. HEMOPHILIA-LIKE DISEASE FOLLOWING PREGNANCY WITH TRANSPLACENTAL TRANSFER OF AN ACQUIRED CIRCULATING ANTICOAGULANT

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(INTRODUCED BY C. J. WATSON, M.D., PH.D.)

An acquired hemorrhagic disease which appeared in a woman three months after her first normal pregnancy has been studied from the time of its onset until recovery two and one-half years later. The venous clotting time was markedly prolonged, and the prothrombin consumption was minimal. Bleeding time, prothrombin time, prothrombin concentration, platelet count, clot retraction, tourniquet test, and plasma fibrinogen concentration were all within normal range. There was no evidence of parenchymal liver dysfunction. The lack of response of the clotting abnormality to fresh blood or plasma led to the suspicion of a circulating anticoagulant which was demonstrated by the delaying effect of the patient's blood on the coagulation time of normal blood. Cohn's Fraction I containing active antihemophilic globulin had no effect on the patient's clotting mechanism even when used in high concentration. The anticoagulant was therefore believed to inhibit the action of the antihemophilic globulin (thromboplastinogen). The patient delivered a second child fifteen months after the onset of the disease, and the anticoagulant could be demonstrated in the baby's blood during its first two and one-half months of life. So far as it can be determined, this is the first report of transplacental transfer of an acquired anticoagulant. The appearance of this inhibitor during pregnancy, its transplacental transfer, and the analogy of this disease to hemophilia which has become resistant to transfusions strongly suggest an immunological mechanism in the development of the anticoagulant. The patient recovered sixteen months after x-ray sterilization. It is not possible to state, however, if this therapeutic procedure had any causal relationship with the return of the coagulation mechanism to normality.

## 38. THE ELASTIC PROPERTIES OF THE LUNG IN PULMONARY EMPHYSEMA

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It is generally agreed that the principal physiologic defect in pulmonary emphysema is an alteration in the elastic properties of the lung. Study of the elasticity of the lung in this disease has been hampered by the danger of measuring intrapleural pressure. In the present study this difficulty was avoided by using intraesophageal pressure as a measure of intrathoracic pressure. Intraesophageal pressure was measured with a specially designed balloon and a differential strain gauge. Comparison of intraesophageal pressure with intrapulmonic pressure and with intrapleural pressure demonstrated that it was an accurate method of measuring changes in intrathoracic pressure.

Measurements were made of changes in static intrathoracic pressure with varying degrees of distention of the lung in normal subjects and in patients with pulmonary emphysema. From these measurements pressure volume diagrams were constructed in order to define the elastic properties of the lung. The curves obtained were sigmoid in character both in the normal subjects and in the patients with pulmonary emphysema.

The functional residual volume is greatly increased at the expense of the inspiratory reserve capacity in pulmonary emphysema. The total lung volume

remains approximately normal. The chief difference in the pressure volume curves between normal subjects and patients with pulmonary emphysema lies in the relationship of the curve to the total lung volume. The relationship of this curve to the functional residual volume remains approximately the same. The result of this alteration in pressure volume relationships is that the elastic forces of the lung are less at any given degree of distention of the lung in patients with pulmonary emphysema than in normal subjects. Inasmuch as the functional residual volume of the lung is determined by a balance between the elastic forces of the lung and the elastic and gravitational forces of the chest wall, it seems likely that the increase in functional residual volume in pulmonary emphysema is largely determined by an alteration in the elastic properties of the lung.

## 39. OBESITY AND IMPAIRED CARBOHYDRATE METABOLISM IN THE "PREDIABETIC" MOTHERS OF LARGE INFANTS

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Persons destined eventually to develop diabetes mellitus are frequently obese. It has been reported that infants born to obese women are often unusually heavy. It is therefore possible that the excessive weight which frequently characterizes infants born to women destined later to develop diabetes is related solely to maternal obesity. However, there have been postulated other theoretical explanations for the large infants, including maternal pituitary dysfunction associated with the eventual development of diabetes in the mother.

In order to elucidate the part played by maternal obesity, we have reviewed the records of 12 pregnancies which occurred in 10 women who weighed over 190 pounds in the third trimester and who were first recognized as suffering from diabetes six to twenty-two years later. The average weight of the 12 infants was 4,373 Gm., significantly greater than the weight of infants born to two control groups of obese women.

Hence, while maternal obesity is often associated with and may somehow be related to the birth of large infants to women manifesting frank diabetes years later, it seems probable that there are other important associated maternal characteristics. We have records of the performance of a glucose tolerance test upon 2 other "prediabetic" mothers of the type described above, during their childbearing era. This procedure revealed impairment of carbohydrate metabolism which preceded the recognition of frank diabetes by many years.

## 40. EFFECTS OF CORTISONE ON THE REMOVAL OF INDIA INK BY THE RETICULOENDOTHELIAL SYSTEM

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The use of cortisone in the treatment of patients with pneumococcic pneumonia has resulted in a persistent bacteremia. In cases of tuberculosis the use of this compound has caused, in some cases, rapid spread of infection. This has suggested that one of the effects of this drug might be temporary suppression of the phagocytic activity of the reticuloendothelial system. This study was undertaken using India ink as particulate matter to see whether the disappearance of this material from the peripheral blood stream could be influenced by cortisone therapy.

A Klett-Summerson colorimeter was used as a densimeter with a 660 Angström's unit filter to remove the hemoglobin color. Standard curves were determined using known dilutions of India ink. Baseline curves were then determined by injecting dogs with one milliliter per kilogram of body weight of a 50 per cent solution of India ink. Control samples were obtained before ink injection. This was injected slowly over a five-minute period. Blood samples were then taken at five-minute intervals for the first half hour and at ten-minute intervals for the second half hour. A final blood sample was taken at seventy-five minutes after injection.

Six dogs were used in the experiment. Initially, five dogs were treated with 50 milligrams of cortisone and then given 18 milligrams intramuscularly for five days. At the end of this time disappearance curves were repeated. Approximately one month later, the same animals were given 100 milligrams of cortisone intramuscularly daily for five days. Disappearance curves were again determined.

One dog was treated with 25 milligrams of cortisone intramuscularly daily for four weeks with the determination of the disappearance of India ink at the end of the one-month period. The results showed that consistent disappearance curves for India ink could be determined in the same animal by the colorimeter technique. However, there was marked variation between animals which was not explainable on the basis of size, sex, health, or state of nutrition of the animal. Cortisone did not appear to have any effect on the phagocytosis of India ink under these experimental conditions.

## 41. CIRCULATORY CHANGES IN ACUTE MYOCARDIAL INFARCTION

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(INTRODUCED BY N. C. GILBERT, M.D.)

The cardiac output, arterial pressure, venous pressure, circulation time, plasma volume, and arterial hematocrit were determined within forty-eight hours after the onset of an acute myocardial infarction in fourteen patients. Cardiac output was measured by the dye dilution technique and plasma volume with Evans Blue dye.

The cardiac index (output per minute per square meter of surface area) was usually low and the total peripheral resistance elevated. As the heart rate was generally increased, the stroke volume was particularly low. The average cardiac index was 2.0 L./min.M.$^2$, and the average total peripheral resistance 2,150 dynes cm.$^{-5}$ sec. The venous pressure was elevated in four of seven patients with manifest congestive failure. It was above normal in two patients without obvious heart failure. In all but one patient with clinical congestive failure, the cardiac index was below 2. The circulation time was inversely related to the cardiac index. Two patients with severe shock had very low cardiac indices, increased total peripheral resistances, prolonged circulation times, and plasma volumes within the normal range. In general, these results agree with those previously reported by ourselves and others.

There appeared to be some correlation between an elevation of the venous pressure and both a lowering of the plasma volume ($r = 0.45$) and an increase of the hematocrit ($r = 0.65$). These findings suggest that an initial elevation of venous pressure in acute heart failure is involved in shifting fluid from within the vascular tree to the interstitial space and is a factor in the formation of cardiac edema. No relationship could be shown between a fall of the cardiac index and an increase in venous pressure, however.

Studies are being conducted with these methods to determine the therapeutic value of measures directed toward restoring normal arterial pressure in patients with myocardial infarction and shock.

## 42. A NEW METHOD FOR THROMBIN ASSAY: ITS APPLICATION TO THE STUDY OF THROMBIN FUNCTION

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The coagulation of fibrinogen by ninhydrin was first observed by Chargaff and Bendich. We have observed that when bovine fibrinogen is first incubated with small amounts of bovine thrombin, its subsequent coagulation by ninhydrin is markedly accelerated. These minute amounts of thrombin which accelerate the ninhydrin coagulation of fibrinogen are themselves incapable of clotting fibrinogen over several hours of observation. By appropriate control of fibrinogen and ninhydrin concentrations, pH, and temperature, thrombin concentrations as small as $5 \times 10^{-5}$ (0.00005) N.I.H. units can be assayed by this procedure, using either a nephelometric method or visual clot formation.

The method has been utilized in studying certain body fluids. Heparin does not affect the ninhydrin clotting time when added to fibrinogen-thrombin mixtures. If, however, small amounts of serum or plasma are present, marked retardation of coagulation occurs.

Human and dog sera which contain no thrombin when measured by conventional methods, if incubated with fibrinogen, will accelerate the ninhydrin coagulation of fibrinogen. This serum accelerating factor increases on storage of the serum at 5° C., until the serum is spontaneously capable of clotting fibrinogen. Since thrombin adsorbants remove this fraction from the serum, while those affecting labile factor and serum prothrombin accelerator globulin have no effect on the reaction, and because of other evidence to be presented, we believe the serum factor to be thrombin.

Fresh oxalated human plasma contains little of the factor which accelerates the coagulation of fibrinogen by ninhydrin, but small amounts of thrombin, or thrombin-like material are measurable in aged plasma.

As a further application of this method, we have studied some of the basic aspects of the conversion of fibrinogen to fibrin. The data obtained would indicate that fibrinogen probably undergoes a series of successive stages prior to its conversion to fibrin. We have also been able to establish that no thrombin is consumed in the early phases of thrombin action on fibrinogen. Moreover, although thrombin itself is thermolabile at 37° C., incubation with fibrinogen protects thrombin from this destructive action.

## 43. EXPERIENCES WITH A NEW ORAL MERCURIAL DIURETIC

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CHICAGO, ILL.

Mercurial diuretics are being extensively used in cases with congestive heart failure and decompensated cirrhosis of the liver. Since many patients rely on mercurial diuretics for maintenance of their "water equilibrium," and since, to date, most of these diuretics have to be given by the parenteral route, a search for an effective oral mercurial diuretic has been going on.

This report is based on our experiences with a new mercurial diuretic (Neohydrin) given orally to 48 patients.

Nineteen patients who entered the hospital with congestive heart failure received, in addition to a low salt diet and digitalis, 4 to 6 tablets daily for four to ten days (average, seven days). The benefited patients showed lessening of dyspnea, orthopnea, and cough, with decrease in weight, size of liver, and edema, and increased urinary output. Two patients had to stop the medication because of side effects. Of the remaining 17, 8 compensated and 3 improved, 6 had little effect, and to them parenteral diuretics had to be given. There was no increase in the nonprotein nitrogen (in 8), and no decrease in sodium or chloride (in 5 and 6 cases, respectively) in whom these determinations were repeated. Mild nausea was present in 5, headache and pruritus in one, and diarrhea in one.

Twenty-nine patients from the cardiac clinic, who received previously 2 c.c. of mercuhydrin weekly, were given from 1 to 6 tablets (average 3.5) daily for up to one hundred twenty-seven days. The results—measured by maintenance of compensation—were good in 23 patients and fair in three. One patient could not take the medication, and two had to be restarted on parenteral diuretics. Ten of the 28 patients showed some side effects but not enough to stop the medication (nausea in 10, two of whom had also headache and 3 mild pruritus). These preliminary observations would suggest that this oral mercurial diuretic might be a valuable substance to replace parenteral diuretics in patients who require continuous diuretic medication.

## 44. INHIBITION BY HEPARIN OF THE LOCAL AND GENERALIZED SHWARTZMAN REACTIONS

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The earliest morphologic alteration in the development of bilateral cortical necrosis of the kidneys, which is the identifying lesion of the generalized Shwartzman reaction, is the occlusion of glomerular capillaries by homogeneous eosinophilic material with the staining properties of fibrin (or fibrinoid). In the local Shwartzman reaction of the skin, Stetson has shown that dermal hemorrhagic necrosis is preceded by the occurrence of platelet-leukocyte thrombi within capillaries and venules.

The question as to whether such vascular occlusions are causally related to the development of renal or dermal necrosis, or are secondary events in the Shwartzman phenomenon, would be clarified by information concerning the effect on the phenomenon of anticoagulant agents.

To this end, the effect of heparin was studied. Rabbits were given intravenous injections of heparin, in a dosage of 35 mg. per kilogram, at various times before and after the provocative intravenous injection of meningococcal toxin. The following results were obtained: heparin completely prevented the development of bilateral cortical necrosis of the kidneys in rabbits subjected to the generalized Shwartzman reaction by two successive intravenous injections of meningococcal toxin. Moreover, the same lesion, which occurs following a *single* injection of toxin in cortisone-treated rabbits, or in rabbits with reticuloendothelial "blockade" produced by thorotrast or trypan blue, was prevented in these circumstances by heparin.

The dermal Shwartzman reaction, produced by preparing an intradermal injection of meningococcal toxin followed one day later by a provoking injection by vein, was similarly prevented by heparin. Also, the "primary" hemorrhagic skin reaction caused by a single intradermal injection of toxin in animals treated with cortisone, thorotrast, or trypan blue was prevented by heparin.

Heparin did not influence the capacity of an intravenous injection of meningococcal toxin to produce ploymorphonuclear leukopenia, nor did it modify the lethal effect of toxin in rabbits.

The findings lend support to the view that the local and generalized Shwartzman reactions are due to an initial interruption of blood flow through the involved tissues through a disturbance of the coagulation mechanism.

## 45. COMPARISON OF THE THERAPEUTIC EFFECTS OF ALKYL PHOSPHATES IN MYASTHENIA GRAVIS

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(INTRODUCED BY WILLIAM C. LEVIN, M.D.)

The treatment of myasthenia gravis with the alkyl phosphates, tetraethyl pyrophosphate (TEPP) and octamethyl pyrophosphoramide (OMPA) has been evaluated. It was the purpose of this study to determine the effects of such therapy on 27 myasthenic patients. Seventeen case studies on each drug were analyzed as to their anti-cholinesterase activity while being withdrawn from neostigmine.

Duration of disease in patients receiving TEPP varied from six weeks to seventeen years. Daily oral dosage averaged 14.2 milligrams. Maximum average serum cholinesterase activity was 5.8 per cent, and red blood cell cholinesterase activity was 9.93 per cent of normal. The clinical condition was greatly improved in 8 and moderately improved in 5 cases. One patient had no response, and 3 had died in myasthenic crises while on TEPP.

Duration of disease in those receiving OMPA varied between seven months and eleven years. Average daily oral dose was 22.2 milligrams. Maximum average serum cholinesterase activity was 18.4 per cent, and red blood cell cholinesterase was 6.8 per cent of normal. Clinical condition was greatly improved in 5 and moderately improved in 8 cases. All showed improvement, but 4 patients have died, 2 of whom left the hospital against medical advice.

Of 7 patients who have received both drugs, 5 felt stronger on OMPA and 2 while on TEPP. All prefer the uniform stability and prolonged action of OMPA, and, because of the predominately peripheral site of action, it lacks the serious central nervous system toxic manifestations of TEPP.

It is concluded that cholinesterase levels are of little value in the clinical regulation of alkyl phosphate drugs in myasthenia gravis. We prefer OMPA to TEPP because its end point of therapeutic effect is abdominal cramping and diarrhea, and not unconsciousness and convulsions.

## 46. THE EFFECT OF HEPARIN ON THE FATE OF INTRAVENOUSLY ADMINISTERED FAT EMULSIONS

M. I. GROSSMAN, M.D., CHICAGO, ILL.

When synthetic fat emulsion is added in vitro to plasma from an animal which has received heparin, clearing of the turbidity takes place. This fall in turbidity can be measured by changes in light transmission in a spectrophotometer, and this has been used as the basis for a quantitative method for determining the concentration of the lipemia-clearing factor in plasma. In rats, increasing doses of heparin up to 0.5 mg. per kilogram intravenously produce increasing lipemia-clearing activity of the plasma; higher doses cause no further increase. No lipemia-clearing activity is found in the plasma of fasting rats. When synthetic fat emulsion and plasma from heparinized animals are mixed

and incubated in vitro and then lyophilized, the added fat cannot be extracted with cold chloroform, suggesting that the fat has become protein bound. Simultaneous intravenous administration to rats of heparin and fat emulsion results in a greatly enhanced rate of disappearance of fat from the plasma, thus heparin not only clears the turbidity, but also increases the rate of removal of fat from the blood. The uptake of fat by the spleen after intravenously administered fat emulsions is decreased by simultaneous administration of heparin. Simultaneous administration of heparin and fat emulsion does not cause significant hemolysis, as is evidenced by the fact that bilirubin output in the bile is not increased. However, when blood drawn from an animal that has received fat emulsion plus heparin is allowed to stand for several hours at room temperature, intense hemolysis occurs.

## 47. HEPARIN TOLERANCE WITH OTHER LABORATORY TESTS AS INDICATORS OF CLINICAL ACTIVITY

### Special Reference to Heparin in Acute Phase Reactions

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(Introduced by Lewis Thomas, M.D.)

The present study was based on the observation that hearts of children who were victims of rheumatic fever more consistently presented atypical tissue mast cells than hearts of controls in the same age group (Hamilton and Syverton: *Am. J. Path.* **26**: 705, 1950). The loss of granules from these cells was interpreted as evidence of liberation of heparin; linearly massed nuclear chromatin (in these cells) was characteristic of Anitschkow cells. Streptococcal-infected monkeys developed similarly prominent atypical cardiac mast cells when they were treated with antiheparins. These findings coupled with the reported increase in heparin tolerance during episodes of active rheumatic fever (Abrahams, Glynn, and Loewi: *Clin. Sc.* **10**: 1, 1951) led us to formulate this working hypothesis: that the fundamental change which underlies activity in rheumatic states logically may be based upon lack of adequate available heparin at sites of tissue damage.

This program has been carried on throughout the Greater Kansas City area in close association with various clinical groups. Two hundred patients were under observation and an equal number of heparin tolerance tests performed. A battery of tests has been designed to ascertain the nature, intensity of activity, and possible characteristic patterns of reaction in active rheumatic fever and certain other pathologic states. The tests are: (1) heparin tolerance, (2) diphenylamine purple reaction (*J. Clin. Investigation* **30**: 781, 1951), (3) antistreptolysin (ASO) titers, and (4) Weltmann reaction (serocoagulation).

Heparin tolerance was increased in 36 individuals (demonstrated through shortened clot delay by fixed amount of heparin). This was most marked during the acute phase of rheumatic state. As patients improved it appeared to reverse to normal before other tests. Other characteristics of the pattern during episodes of acute activity include: elevated diphenylamine reaction, exaggerated increase in ASO titer, and positive Weltmann (shift to left in acute phase).

There was an increase in heparin tolerance in nephrotic phase of renal disease, toxemia of pregnancy, and in early burns and keloids. These diseases

were not characterized by the pattern of rheumatic state. The diphynlamine reaction was relatively low in nephrosis. Heparin therapy yields certain interesting and beneficial responses.

## 48. THE EFFECTS OF DEXTRAN IN SUBJECTS WITH NORMAL BLOOD VOLUMES AND IN SUBJECTS AFTER BLEEDING

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(INTRODUCED BY EDMUND B. FLINK, M.D.)

Dextran is a macromolecular plasma volume expander. Although numerous clinical reports have appeared in the literature, measurements of the effects on plasma volume and plasma protein synthesis have been few.

One liter of 6 per cent dextran was administered intravenously to 8 normal men after the rapid removal of one liter of blood. The plasma volume, hematocrit, plasma protein, and plasma and urinary dextran concentrations were determined one, six, twelve, and twenty-four hours after the administration of dextran. Control observations were made prior to the venesection.

The mean increases in plasma volume were 1,020, 820, 700, and 860 ml. at one, six, twelve, and twenty-four hours, respectively. The mean increase in circulating protein at the stated periods was 3.3, 15.7, 19.2 and 30.6 Gm. The plasma dextran concentration was 1,160 mg. per 100 ml. at one hour, and decreased progressively to 431 mg. per 100 ml. at twenty-four hours. The total circulating dextran was 41.5 Gm. at one hour and decreased to 14.9 Gm. at twenty-four hours. Approximately 25 per cent of the administered 60 Gm. of dextran was excreted in the urine in one hour, and approximately 58 per cent was excreted in twenty-four hours. At the end of twenty-four hours about 17 per cent of the administered dextran could not be accounted for, and is assumed to have been present extravascularly.

One liter of dextran was also administered to 10 convalescent male patients without cardiovascular or renal disease, and similar measurements were carried out. The mean increase in plasma volume was 775 and 534 ml. at one and six hours, respectively. The plasma dextran concentration and urinary dextran excretion were similar to those cited above.

These experiments indicate that dextran is a satisfactory plasma volume expander in oligemic states. It is noteworthy that dextran does not inhibit protein synthesis. As dextran leaves the circulation, plasma protein is progressively added and hemodilution continues. In the experiments on subjects who were not bled, it was calculated that 1 Gm. of dextran binds 21.2 ml. of water.

## 49. THE DELAYED TOXICITY OF RADIUM DEPOSITED IN THE SKELETON OF HUMAN BEINGS

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A residuum of individuals who were injected with solutions of radium for therapeutic purposes between twenty and thirty years ago exists in this country. This group is of fundamental importance in quantitating the neoplastic response of the skeleton to the alpha particle-emitting, bone-seeking radioelements and in establishing permissible levels of body content.

Data derived from experiments done on small laboratory animals are not readily extrapolated to man because of marked differences in life span, variations in the geometry of the biological systems studied, and differences in mineral metabolism. Inherent in a statistically unbiased analysis of the data derived from human material is the requirement that the groups studied be not unduly weighted with individuals seen by physicians *first* because of the appearance of clinical symptoms. To this end the authors undertook to seek out and clinically evaluate individuals in the Chicago area who had received radium many years ago and who were selected, in so far as possible, for this reason alone. Several of these individuals, moreover, were unaware that they had been given radium.

The present study consists of clinical, roentgenographic, and physical studies done on twenty-three persons who contain radium and fulfill the above criterion with but six exceptions. These studies extend the data derived from fourteen patients at the Elgin State Hospital and presented one year ago.

Analyses of roentgenograms and physical data indicate the presence of diagnostic bone lesions in individuals retaining as little as 0.4 $\mu$g of radium, with a correlation between ascending levels of radium retention and the frequency and severity of the roentgenographic lesions representing bone resorption and necrosis. The incidence of neoplasia in this series is rare enough to make impossible at present correlations between total-body content and tumor induction. Further experience in the interpretation of roentgenograms makes it possible to define and recognize early bone lesions in the asymptomatic individual.

## 50. PHYSIOLOGICAL EVALUATION OF THE RESULTS OF MITRAL VALVE SURGERY

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(INTRODUCED BY GORDON B. MYERS, M.D.)

Eleven patients with mitral stenosis have been studied by venous catheterization before and from two to sixteen months after mitral valve surgery to determine the early and late hemodynamic effects of the operation.

Prior to operation mean pulmonary capillary and artery pressures were elevated in each patient to levels ranging from 19 to 36 mm. Hg and 28 to 68 mm. Hg, respectively. Cardiac index varied from 1.7 to 3.9 L./min./M.$^2$, but was decreased below 3.0 L./min./M.$^2$ in eight patients. There was a marked decrease in calculated mitral orifice area to below 1.1 cm.$^2$ in all patients.

Repeat hemodynamic studies from two to eleven months after surgery showed the following results: pulmonary capillary pressure had decreased from 4 to 24 mm. Hg in eight patients, was unchanged in two, and had increased in one; mean pulmonary artery pressure showed a reduction of from 8 to 21 mm. Hg in five patients, was not significantly changed in five, and increased in one; cardiac index increased from 0.2 to 1.7 L./min./M.$^2$ in five patients, did not change in two, and decreased from 0.5 to 0.8 L./min./M.$^2$ in four; and calculated mitral orifice area increased from 0.2 to 0.7 cm.$^2$ in seven patients and changed by less than 0.2 cm.$^2$ in four.

Three patients who had been studied four, seven, and two months after operation had a second catheterization performed after intervals of seven, nine, and thirteen months, respectively. Two showed continuing hemodynamic improvement with further slight increases in calculated valve area. In the third patient the calculated valve area was 1.1 cm.$^2$ prior to surgery, increased

to 1.8 cm.$^2$ two months after surgery, and narrowed to 1.0 cm.$^2$ thirteen months later. This, together with the recurrence of congestive failure, was attributed to reclosure of the commissures. In the remaining ten patients there was good correlation between hemodynamic and clinical data. There was clinical improvement in six patients associated with significant increases in mitral orifice area, and only slight or transitory improvement in four patients having no measurable change in valve size.

## 51. ELECTROCARDIOGRAPHIC EXERCISE TOLERANCE TESTS IN OLD MYOCARDIAL INFARCTION

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In the course of evaluating the physical fitness of patients at the Cleveland Heart Work Classification Clinic, exercise tests (Masters two step) were performed safely on 55 consecutive patients with old myocardial infarction.

The exercise tolerance tests of 25 additional patients with old myocardial infarcts and debilitating angina (on whom later the Beck aorta-coronary sinus anastomosis was performed) provided information about ECG changes in patients with proved severe coronary arteriosclerosis.

The magnitude, direction, and sense of the QRS, ST junction, and ST and T vectors were determined before and after exercise, in addition to the usual method of analysis. Criteria for positive tests included: disturbance of intraventricular conduction, excessive sinus tachycardia, increase or appearance of ectopic beats or rhythms, reversal of T waves without QRS change (significant change in T vector position and magnitude), appearance, increase, or shift of ST junction vector (ST segment depression more than 1 mm. in 2 limb leads and chest leads), and clinical angina. The vectoral method of Grant simplified the evaluation of the test.

*Results.*—Twenty-four cases had unequivocally positive tests: pbs, 4; bundle branch block, 1; STT changes, 11; clinical symptoms alone, 2; STT and symptoms, 6. Twenty-four cases had unequivocally negative tests. Seven cases were classified as equivocal: one had no changes but experienced severe dyspnea; one had digitalis medication, possibly invalidating increase of ST vector; 5 cases had borderline increase or decrease of T vector associated with slight QRS shifts. Similar features in each group included age distribution (38 per cent less than 50 years) at least three months between exercise tolerance test and last clinical infarct, low incidence of congestive failure (3 in total series), generalized arteriosclerosis (76 per cent), and predominance of mesomorphy. The group with negative exercise tolerance was characterized by a lower incidence of history of angina pectoris, better functional and therapeutic classification, less ventricular hypertrophy, and a higher incidence of nontransmural infarcts. Twelve patients had ventricular aneurysms. Of these 6 had positive tests, 3 negative, and 3 equivocal.

*Conclusions.*—Exercise tolerance tests have been performed safely in patients with old myocardial infarcts, of which 43.6 per cent were negative, 43.6 per cent were positive, and 12.8 per cent equivocal. The results of these tests, integrated with other findings, have been helpful in making practical work recommendations for these patients.

## 52. RESPONSES TO CORTICOTROPIN (ACTH) AND CORTISONE IN THROMBOCYTOPENIC STATES

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During the past twenty-two months ten patients with thrombocytopenic purpura of varied etiology have been treated with corticotropin or cortisone or a combination of the two. Seven patients were considered to have the idiopathic form of the disease. In two patients drug hypersensitivity was suspected, and one patient developed thrombopenic purpura in the course of acute disseminated lupus erythematosus.

The three patients with thrombocytopenic purpura attributed to drug reactions and lupus were treated with corticotropin with prompt clinical and hematologic remissions which have persisted twenty-two months, sixteen months, and ten months, respectively. Three of the seven patients in the idiopathic group received corticotropin alone, one with no response, one with prompt clinical as well as platelet response, and one with prompt symptomatic relief and gradual hematologic improvement.

Three patients received cortisone only. One responded promptly with disappearance of purpura and rise in platelets. Two responded clinically but showed no rise in platelets over periods of four months and five weeks, respectively.

One patient treated with corticotropin intravenously for one week experienced remission of all vascular manifestations but failed to exhibit increase in platelets during that period or during a subsequent fifty-day course of cortisone.

It appears that corticotropin (ACTH) and cortisone may exert a beneficial effect on capillary fragility and the bleeding phenomena of thrombopenic purpura not necessarily associated with an increase in circulating thrombocytes. Although these agents appear useful in the management of certain thrombopenic states, they fall short of fulfilling their earlier promise in many instances.

## 53. OPERATIVE AND POSTOPERATIVE CONTROL OF BLOOD PRESSURE IN PATIENTS WITH PHEOCHROMOCYTOMA

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Although surgery offers the only means of a cure for pheochromocytoma, the operative and postoperative mortality rates, as reported in the literature, are 26 per cent. The most common cause of death is severe hypotension occurring within seventy-two hours after operation. However, death due to severe hypertension, pulmonary edema, or cardiac arrhythmia is not uncommon during operation.

The release of large amounts of epinephrine and norepinephrine during operation accounts for the severe hypertension, and may result from palpation or traction upon the tumor. Hypotension and the shocklike state which develop postoperatively appear to be due to sudden lack of circulating vasopressor substances to which the patient has become accustomed.

The above complications can be prevented by use of appropriate vasodepressor and vasopressor agents during and after operation. An indwelling plastic needle is used for the prompt intravenous administration of drugs and

fluids. As wide fluctuations in blood pressure may occur rapidly, the blood pressure is determined at intervals of one to two minutes.

Marked elevations in blood pressure are controlled by regitine or piperoxan hydrochloride given intravenously in doses of 5 to 20 mg. These short-acting adrenergic blocking agents also are of value in detecting the presence of multiple tumors at the time of operation in patients with persistent hypertension.

If hypotension develops postoperatively, norepinephrine or a combination of norepinephrine and epinephrine in 5 per cent solution of glucose is administered slowly by continuous intravenous drip. Vasopressor therapy may be necessary for the first forty-eight to seventy-two hours postoperatively. The amount of norepinephrine and epinephrine required has varied from 4 to 40 mg. in our patients. Constant supervision and frequent readjustment of the rate of administration are necessary. The amount of drug is gradually decreased, and its use is discontinued when the blood pressure is stabilized. Subcutaneous infiltration of norepinephrine must be avoided, as marked necrosis at the site of injection may occur.

Within the past year we have studied nine patients who had single or multiple pheochromocytomas, and with the above regimen we have observed no serious operative or postoperative complication.

## 54. REFRACTORY MACROCYTIC ANEMIA WITH DEFECT IN VITAMIN $B_{12}$ BINDING AND WITH RESPONSE TO NORMAL PLASMA

DANIEL L. HORRIGAN, M.D. (BY INVITATION), AND ROBERT W. HEINLE, M.D., CLEVELAND, OHIO

In vivo studies in this laboratory and in vitro bone marrow culture studies by others have suggested that vitamin $B_{12}$ is utilized for hematopoiesis in a bound form. It was postulated, therefore, that a deficiency in binding of the vitamin might result in "refractory macrocytic anemia."

A patient was observed who has had unexplained anemia for eighteen years which did not respond to crude liver extract given intramuscularly every week nor to iron orally. Free HCl was present in fasting gastric contents. Study of hematologic remissions and relapses in this patient during the past year have provided data relative to the role of $B_{12}$ binding in the utilization of the vitamin for hematopoiesis. Hematologic findings during relapse included macrocytic anemia, reticulocytosis, and normoblastic bone marrow hyperplasia. Remissions have been produced by a single intramuscular dose of 90 $\mu$g of vitamin $B_{12}$ or by the intravenous administration of 250 ml of human plasma. Subsequent to administration of either, there was a rise of hemoglobin from 9 to 12 Gm. per 100 c.c. of blood, and of the hematocrit from 31 to 40 per cent. In either instance, remission was not maintained either with vitamin $B_{12}$, 30 $\mu$g intramuscularly every one to two weeks, or with oral folic acid, 10 mg. daily.

Intramuscular administration of 0.5 $\mu$g of $Co^{60}$-labeled vitamin $B_{12}$ resulted in the urinary excretion of 60 per cent of the administered dose within twenty-four hours. (Normal persons excreted 0 to 10 per cent under similar conditions.) After giving normal plasma, the urinary excretion of the same dose of the labeled vitamin decreased to 24 per cent. Uric acid in the erythrocytes was elevated during relapse and decreased to normal with remission. No evidence of increased blood destruction was demonstrated.

These observations suggest that (a) the anemia in this subject results from $B_{12}$ deficiency caused by failure of proper binding of the vitamin, and

(b) normal plasma contains a factor which corrects this defect. This factor may be (a) binding substance itself, (b) a substance necessary for the combination of $B_{12}$ and the binding substance endogenously, or (c) amounts of the vitamin in bound form sufficient to promote hematopoiesis.

## 55. THE COMPARATIVE EFFECTS OF ACTH, CORTISONE, AND ASPIRIN ON THE ACUTE COURSE OF RHEUMATIC FEVER

Major Harold B. Houser (MC), Army of the United States, and Captain Ernest J. Clark and Captain Bertrand L. Stolzer (MC), United States Air Force, Francis E. Warren Air Force Base, Wyoming

(Introduced by Charles H. Rammelkamp, Jr., M.D.)

As part of a cooperative study co-ordinated by the American Council on Rheumatic Fever, the comparative effects of ACTH, cortisone, and aspirin on acute rheumatic fever have been studied in a series of 152 young adult airmen hospitalized at Francis E. Warren Air Force Base, Wyoming.

Physical examinations were done daily for nine weeks on each patient by one of three observers. Laboratory procedures were performed at frequent and standard intervals. Therapy, initiated during the first week of illness in the majority of patients, was administered for six weeks. The daily dose of ACTH was 120 mg. initially and was reduced at specified intervals so that at the beginning of the sixth week the dose was 20 mg. daily. Intramuscular cortisone was reduced gradually from 300 mg. on the first day to 50 mg. per day during the sixth week. Aspirin was reduced during the first week from 1 grain per pound per day to a maintenance dose of ½ grain per pound per day.

Aspirin was most effective in relieving symptoms and signs of joint involvement. Fever subsided most promptly following aspirin therapy, although there was no marked difference among the groups in the incidence of fever after the fifth day of treatment. ACTH was most effective in returning the erythrocyte sedimentation rate to normal. Abnormal A-V conduction occurred with similar frequency among the three groups, but the duration of abnormalities was slightly longer in the aspirin group. There was no marked difference among the three groups in the appearance or disappearance of murmurs or of other signs of acute carditis. Aspirin was discontinued in two patients because of severe hypoprothrombinemia. Appearance of acute psychoses necessitated discontinuance of ACTH in two patients. After treatment, clinical or laboratory relapses occurred in the majority of patients in all groups. With few exceptions, these relapses subsided promptly.

It is concluded that no drug employed showed a marked advantage over the others in the treatment of rheumatic fever.

## 56. THE ULTRASONIC VISUALIZATION OF SOFT TISSUE STRUCTURES AND DISEASE PROCESSES

Douglass H. Howry, M.D., Denver, Colo.

(Introduced by Joseph H. Holmes, M.D.)

Ultra high-frequency mechanical waves are being utilized to make soft tissue structures and disease processes such as carcinoma easily visible. A very short pulse of ultrasonic waves is focused into a pencil-like beam which is caused to travel through tissue structures. The resulting echoes from tis-

sue interfaces are then electronically amplified and produced as bright spots on a television-like tube. By rapid scanning of the sound beam and synchronous movement of the bright spots produced on the tube face, a visually continuous picture is produced. This has been applied to visualization of benign and malignant tumors. Pictures will be shown from pathologic specimens of a cyst of the kidney, metastatic lesions of the liver, and a carcinoma of the breast with metastases to the lymph nodes. The carcinoma of the breast showed well on the "Somagram" despite the fact that it was a nonpalpable lesion. Application to living structures has produced no sensation or damage, while an excellent demonstration is afforded of the blood vessels, nerves, tendons, and fascial planes of an upper arm and forearm. The potential application of the unit to the detection and differential diagnosis of tumors in areas such as the thyroid and kidney and to fluid accumulations in pleura and pericardium will be discussed. The unit also has potential application for study of the thickness of the right ventricle and demonstration of cardiac motion. The theoretical and practical limitations of this method of examination will be discussed.

## 57. SKIN SENSITIVITY TO HISTOPLASMIN

SAMUEL HYMAN, M.D., AND JEROME F. SICKLEY, M.D.,
CHICAGO, ILL.

(INTRODUCED BY FREDERICK STEIGMANN, M.D.)

Clinically and roentgenologically, histoplasmosis in the active pulmonary stages very closely resembles pulmonary tuberculosis. The patients in Cook County Hospital are generally in the lower social-economic strata, with approximately 50 per cent belonging to the Negro race, and have a high incidence of pulmonary disease. Since Chicago is located in the periphery of the known histoplasmosis area, it was of interest to determine the incidence of possible histoplasmosis infection as determined by a positive skin test.

Accordingly, 600 adult patients, men and women, with various medical diseases, exclusive of tuberculosis, were skin tested. Approximately 10 per cent of these were found to be positive, as measured by an area of induration 5 mm. or larger when read forty-eight hours after an intracutaneous injection of 1:1,000 dilution of histoplasmin.

Seventy patients with moderately and far-advanced pulmonary tuberculosis, all of whom had a positive tuberculin test, were also skin tested for histoplasmosis. Of these patients only 4, or 6 per cent, had a positive histoplasmin reaction.

A preliminary survey of the patients having positive histoplasmin reactions did not reveal any evidence pointing to active histoplasmosis infection. The positive skin test, therefore, seems to indicate a possible infection at some time prior which now is healed.

The differential diagnosis between tuberculosis and histoplasmosis can be aided by a positive histoplasmin skin test in the presence of a negative tuberculin test. When both skin tests are positive, the definite diagnosis must rest on the identification of the causative organism. While the presence of a positive skin test is not definite proof of an active histoplasmosis infection, the 10 per cent incidence of positive skin tests for histoplasmosis would nevertheless suggest that this disease should be considered in the diagnosis of pulmonary diseases in this area.

## 58. CELLULAR AND EXTRACELLULAR FACTORS IN CONGESTIVE HEART FAILURE

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In previously reported balance studies conducted in seven patients during recovery from congestive failure, we demonstrated cellular uptake of potassium and sodium and release of water, and postulated changes in opposite direction during development of failure. Movement of electrolytes out of cells and water into cells during development of failure could reflect a relative increase in cellular osmolarity. Likewise, in a series of untreated cardiac patients in failure the plasma sodium levels were found to be significantly elevated, reflecting a concomitant increase in extracellular osmolarity.

The water and electrolyte content of skeletal muscle biopsied at the height of failure and again after recompensation was determined in eight cases to elucidate further the mechanism of congestive failure. The potassium content rose from an average value of 5.06 to an average of 7.23 meq. per 100 Gm. wet tissue, rising in seven and falling in one case. The chloride content determined in seven cases decreased from an average of 3.8 to an average of 3.1 meq., falling in five and rising in two cases. The sodium content varied considerably and did not change consistently. Calculated extracellular and intracellular partitioning of water was not consistently affected, but in five of seven cases the calculated intracellular potassium concentration rose after recompensation.

These results indicate a cellular deficiency of potassium in congestive failure most likely due to increase in osmotic activity of the cells. It is postulated that the necessity of maintaining high osmolarity in extracellular fluid equal to that in cells during circulatory insufficiency may provide the stimulus for retention of sodium, and the necessity of reducing the toxicity of intracellular fluid may induce retention of water.

## 59. PAROXYSMAL VENTRICULAR TACHYCARDIA TREATED WITH INTRAVENOUS QUINIDINE

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The intravenous administration of quinidine for terminating paroxysms of ventricular tachycardia, although known to be effective, is not generally accepted as safe because serious reactions have been emphasized.

Eleven patients have been treated with 32 intravenous injections of quinidine for 28 attacks of ventricular tachycardia. Twenty-four of the attacks were terminated during or within a few minutes after the injection. Dramatic clinical improvement occurred after termination of 18 attacks. There was an abrupt rise of the blood pressure from shock levels to normal in 10 instances. Eight patients who were critically ill and 3 who were comatose at the time of injection recovered. Electrocardiograms are available showing the abrupt termination of 8 attacks.

Six injections of quinidine were given for 2 attacks in 2 patients in whom there was only initial slowing of the ventricular rate, several hours elapsing before normal sinus rhythm was restored. Therapy failed to terminate at-

tacks in 2 patients, one of whom, moribund at the time injection was begun, died after 130 mg. had been given.

Dosage per injection varied from 130 mg. to 0.65 Gm. Ampules of quinidine lactate or gluconate were diluted to 20 ml. with normal saline, and the rate of injection did not exceed 65 mg. per minute. Continuous auscultation of the heart and the use of a direct writing electrocardiograph during the injection are advised. Injection should be discontinued whenever any change occurs in the ventricular rate or in the quality of the heart sounds or upon the appearance in the electrocardiograms of adverse effects of quinidine.

Serious reactions have not occurred. The death of one patient cannot be unequivocally ascribed to quinidine toxicity, in view of his status at the time injection was begun. It is concluded that if the method as outlined is followed, the intravenous injection of quinidine is a safe and highly effective means for terminating paroxysms of ventricular tachycardia.

## 60. RADIOAUTOGRAPHIC LOCALIZATION OF $NA^{22}$ IN THE RAT KIDNEY

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(INTRODUCED BY WILLIAM B. WARTMAN, M.D.)

Male and female Sprague-Dawley rats on normal and low sodium diets were injected intravenously and intraperitoneally with 5 or 10 $\mu$c of $Na^{22}$ as the chloride. Gross localization of the radiosodium in the rat kidney was then accomplished by taking radioautographs of thin frozen dehydrated sections of kidney. The renal tissue was never allowed to come in contact with water or to reach a temperature above $-40°$ C. from the time it was removed from the experimental animal until it was completely dehydrated and the photographic film exposed. The slide was then stained and compared with the developed negative.

Autographs obtained at any time from four minutes to twenty-four hours following injection of radiosodium in animals on normal or sodium-deficient diets show localization of the isotope to the outer zone of the cortex and the inner zone of the medulla (chief tubular constituent-collecting ducts). The medullary exposure is greater than that observed in any other zone in the kidney. The outer zone of the medulla, which contains the vasa rectae, is appreciably exposed above background only when 10 $\mu$c of $Na^{22}$ are injected.

The exposure produced by radiosodium in the intravascular space is identified by comparing the radiosodium autographs to the autographic picture of the vascular tree of the rat kidney obtained through the use of intravenous $P^{32}$-labeled rat red blood cells.

The interpretation of these autographs will be discussed and correlated with chemical data obtained from these animals.

## 61. THE DISPOSAL OF $I^{131}$-LABELED THYROXINE IN MYXEDEMA, EUTHYROIDISM, AND ARTIFICIAL HYPERTHYROIDISM

PHILIP C. JOHNSON, M.D. (BY INVITATION), AND WILLIAM H. BEIERWALTES, M.D., ANN ARBOR, MICH.

Five adults were given orally less than 0.1 mg. of Na-l-thyroxine labelled with 585 to 924 microcuries of $I^{131}$. The experimental subjects were a classical myxedema following total thyroidectomy with artificial hyperthyroidism induced by desiccated thyroid given in gradually increasing doses up to 25 grains per day; two euthyroids post cholecystectomy with T-tube in place

and persistently clay-colored stools; one untreated primary myxedema; and a euthyroid with emphysema. Quantatitive $I^{131}$ analyses were made on urine specimens every hour for six hours, then on twenty-four hour specimens for six days. Quantitative total fecal $I^{131}$ analyses were made daily for six days. Quantitative total $I^{131}$ assays of bile were performed at the same intervals. Separate bile, stool, and urine $I^{131}$ excretions were plotted in cumulative total microcuries of $I^{131}$ and as per cent of administered dose. Quantitative total blood $I^{131}$ and protein-bound $I^{131}$ determinations were performed two to four times during the six-day interval in 4 of these patients. Quantitative inorganic and "thyroxine" $I^{131}$ paper chromatography was performed on daily twenty-four hour urine specimens for three days in the control euthyroid patient.

The most striking findings were: (a) total six-day $I^{131}$ excretion in urine and stool was similar in magnitude in these myxedematous, euthyroid, and hyperthyroid subjects. (b) In six days the two bile-fistula patients excreted only 7.3 to 10.3 per cent of the administered dose of Na-1-thyroxine $I^{131}$ in an average daily bile volume of 552 to 648 ml. The enterohepatic circulation of thyroxine would appear not so great in these human beings as previously reported in mice. The paucity of biliary excretion of thyroxine $I^{131}$, plus the fact that (c) fecal excretion of thyroxine-derived $I^{131}$ was the same both in bile fistula (24 to 32 per cent of administered Na-1-thyroxine $I^{131}$) and in intact patients (21 to 27 per cent), indicates that thyroxine is present in the feces not because of fecal bile, but either through lack of absorption from gut or active excretion through gut wall. The fact that metabolism of intravenous thyroxine and oral thyroxine is reportedly the same would suggest that the latter explanation is more probable.

Unlike previous work in animals, 25 per cent of urinary $I^{131}$ was found by chromatography to be present in thyroxine fraction the first day, 16 per cent the second, and 54 per cent the third day.

## 62. A STUDY OF ILLNESS IN A GROUP OF FAMILIES: TYPES OF ILLNESS OBSERVED AND INVESTIGATION OF POSSIBLE ENTITIES OF UNKNOWN ETIOLOGY

WILLIAM S. JORDAN, JR., M.D., GEORGE F. BADGER, M.D. (BY INVITATION), JOHN H. DINGLE, M.D., A. E. FELLER, M.D., RICHARD G. HODGES, M.D. (BY INVITATION), AND CHARLES H. RAMMELKAMP, JR., M.D., CLEVELAND, OHIO

A highly selected population of families has been observed to determine the occurrence and behavior of illnesses. During a four-year period, 82 families, comprising a total of 380 individuals, were intensively studied in an effort to measure the true occurrence of deviations from a state of health and to indicate the mechanism and causation of such deviations.

The incidence of total illness was constant at a level of approximately 10 illnesses per person-year. Infections of the respiratory tract accounted for approximately two-thirds of total illnesses, the incidence being constant at 6.5 illnesses per person-year in the 1948-51 period. Gastrointestinal illnesses accounted for about 15 per cent of the total illnesses.

Of 5,825 respiratory illnesses, only 2.8 per cent were identified as bacterial infections. Common respiratory disease comprised the largest group of nonbacterial infections (94.3 per cent of the total), of which the common cold accounted for approximately 68 per cent, or about 43 per cent of all illnesses. Between September 1 and April 20, an average of 15 per cent of the population had a new respiratory illness each week.

One of the objectives of the study is to obtain leads for etiological investigation. Attempts thus far to isolate, in eggs or animals, agents causing common respiratory disease have been unsuccessful. It has, however, been possible to demonstrate in volunteers a transmissible nonbacterial agent inducing acute gastroenteritis. Clinical and epidemiological data regarding the nonbacterial gastrointestinal illnesses suggest that two types of acute infectious gastroenteritis, afebrile and febrile, occur in the families. In cooperation with Dr. Irving Gordon, it has been shown that inoculum from a febrile case (FS) induces illnesses which differ from the afebrile illnesses induced by the Marcy strain, a filterable agent obtained from an institutional outbreak in New York State. Lack of heterologous immunity, variation in incubation period, and the different clinical pictures indicate that one of the types of gastroenteritis observed in families, the febrile type (FS) differs from the Marcy type and presumably represents a new entity.

## 63. HUMAN ASCORBIC ACID REQUIREMENTS IN "DAMAGE" AND ITS RELATIONSHIP TO ADRENOCORTICAL ACTIVITY

ROBERT M. KARK, M.R.C.P., ROBERT E. CHAPMAN, M.D. (BY INVITATION), C. FRANK CONSOLAZIO (BY INVITATION), AND CHARLES NESBY (BY INVITATION), CHICAGO, ILL.

Lund and associates (*Ann. Surg.* **124**: 840, 1946) have shown that urinary and plasma ascorbic acid falls to very low levels in "damaged" surgical patients during the "catabolic phase" of stress, despite daily parenteral therapy with very large (normally saturating) amounts of ascorbic acid. These data are accepted widely as evidence that daily requirements for ascorbic acid in man are increased after surgery and other insults. The demonstration in rats that adrenocortical activity consumed adrenal ascorbic acid during and after "stress" offered a seemingly rational explanation for the so-called increased utilization of ascorbic acid after surgery.

This study deals with the effects of three to eleven days of therapy with adrenocorticotropin or cortisone on: the whole blood plasma, fecal and urinary ascorbic acid, the circulating eosinophils, and the urinary 17-ketosteroid excretion of patients and healthy individuals studied during fixed dietary regimens and fixed analyzed ascorbic acid intakes while living in a metabolic unit. Balances of ascorbic acid in food and excreta and blood levels were measured by Roe's method. Available data indicate that: (a) in classical scurvy, good adrenocortical response to ACTH occurs, as shown by a drop in eosinophils and a rise in 17-ketosteroid excretion. Ascorbic levels in whole blood, urine, and feces do not change, (b) in individuals "saturated" with ascorbic acid, urinary ascorbic acid increased considerably during ACTH or cortisone therapy and during adrenal rebound some days after therapy was stopped. Whole blood, plasma, and fecal ascorbic acid levels did not alter significantly, (c) in "unsaturated" individuals, urinary ascorbic acid levels sometimes increased and sometimes remained unchanged. In no individual was there retention of urinary ascorbic acid as seen in Lund's study. Patients were also studied before and after extensive orthopedic operations. The investigation was designed to mimic those of Lund and associates, i.e., measurements of urinary and blood ascorbic acid were made during postoperative therapy with large amounts of ascorbic acid. In addition to these, studies of urinary 17-ketosteroid excretion and blood eosinophil levels were measured. In a number of patients long-acting ACTH (Wilson) was injected two to three days after operation to observe its effect on the metabolism of ascorbic acid.

The data indicate that in man consumption of ascorbic acid by the adrenal for synthesis of cortical hormone cannot be considerable, adrenocortical activity cannot account for the urinary retention of ascorbic acid seen in surgical damage, a diuresis of ascorbic acid occurs with adrenocortical activity when large amounts of ascorbic acid are ingested.

On the basis of the above investigations, speculations will be made on the requirements for ascorbic acid in the catabolic phase of "damage."

## 64. THE TRANSFER OF SODIUM AND CHLORIDE ACROSS A MEMBRANE PRODUCED IN MAN BY THE APPLICATION OF THE VESICANT CANTHARIDES

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(INTRODUCED BY G. E. BURCH, M.D.)

Blisters were produced on both forearms of 39 subjects by the application of Emplastrum cantharides. The top of the blister was removed and a lucite cup was cemented over the base (10 cm.). Ten cubic centimeters of saline, plasma, or other solutions containing radioactive $Na^{22}$ or $Na^{24}$ or $Cl^{36}$ were placed in the cups and seven 1 c.c. samples removed for analysis of radioactivity and total Na and Cl over a four-hour period. One forearm served as the control for its fellow when variables were introduced. In four experiments $Na^{24}$ was injected intravenously and $Na^{22}$ added to the cups so that transfer of sodium in both directions could be studied. Patients with congestive heart failure were contrasted with control subjects.

From the calculation of data obtained, and comparison of these data with those previously reported using a mercurial diuretic labeled with radioactive mercury, the following observations were made: (a) The behavior of the membranes on the two forearms of an individual would be expected to agree within 10 per cent. The per cent of $Na^{22}$ remaining in the cups of control subjects after four hours was 41.6 ± 4 per cent. (b) Sodium is transferred in both directions across this membrane at the rate of 0.15 meq. per 30 min. per 10 $cm^2$. Sodium does not cross intact skin. Elevation of venous pressure or variation of pH did not influence the movement of sodium. (c) The rate of disappearance of $Na^{22}$ from the cups containing 1.8 per cent NaCl was somewhat more rapid than from cups containing 0.9 per cent NaCl which in turn was slightly more rapid than that distilled from water or isotonic KCl. (d) Chloride disappeared slightly more rapidly than sodium from solutions of plasma, 0.9 per cent NaCl, and distilled water. (e) The rate of transfer of sodium and chloride (0.9 per cent NaCl) was ten to fifteen times that of mercury. When plasma was the vehicle, these differences were magnified indicating protein binding of mercury. (f) The rate of disappearance of sodium from cup fluid (0.9 per cent NaCl) in patients with congestive heart failure did not differ from that observed in control subjects.

## 65. SOME EFFECTS OF HEXAMETHONIUM (C-6) AND 1-HYDRAZINOPHTHALAZINE (HP) ON KIDNEY CIRCULATION

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Glomerular filtration rate (GFR) and effective renal plasma flow (RPF) were measured before and after the intravenous injection of hexamethonium chloride in 12 patients and of 1-hydrazinophthalazine in 7 patients.

Following C-6 (20 to 160 mg.) GFR and RPF either decreased or remained at control levels in patients with malignant hypertension (3), coarctation of aorta (2), nephritis (2), and in one of the two normals. In patients with essential hypertension (3) and in one subject who had normal blood pressure but hypertensive renal vascular pattern during the control period, RPF increased 10 to 75 per cent (average, 44 per cent) following C-6. There was little change in GFR, but filtration fraction (FF) decreased from 17 to 45 per cent (average, 29 per cent) below control values.

After HP (10 to 30 mg.) two normals, one hypertensive and one mild nephritic, had increased RPF 11 to 31 per cent (average, 23 per cent) but no significant GFR change, while the patient with severe nephritis (1), coarctation of aorta (1), and one with hypertension who went into mild shock following the injection had either no increase or a fall in RPF. GFR was lowered in this group. FF fell in all 7 patients.

Apparently the effect of C-6 and HP on renal circulation differs in health and certain diseases. In far-advanced kidney disease, where extensive structural changes have taken place, neither C-6 nor HP increased RPF, nor did they do so if there was too great a decrease in blood pressure or if coarctation of the aorta was present (where the mean head of pressure available to the kidneys is low). In uncomplicated benign arterial hypertension, both drugs caused an increase in RPF without an unfavorable effect on GFR, thus changing the hypertensive renal functional picture toward normal. HP increased RPF in normal subjects, an effect not seen with C-6.

## 66. STUDIES OF HEMODYNAMIC CHANGES IN HUMAN BEINGS FOLLOWING INDUCTION OF HIGH SPINAL ANESTHESIA

### III. The Effects on Cerebral Blood Flow, Cerebral Oxygen, and Glucose Metabolism

Jerome Kleinerman, M.D., Salvatore M. Sancetta, M.D., R. Beverley Lynn, M.D., Robert Stahl, M.D., and Donald B. Hackel, M.D., Cleveland, Ohio

(Introduced by Roy W. Scott, M.D.)

In previous communications we have described the general hemodynamic and splanchnic changes attending high spinal anesthesia in surgical and nonsurgical patients. The present report presents the effects of high spinal anesthesia on the hemodynamics (CH), and metabolism (CM) of the brain in patients not operated upon, and differs from the investigations of others in three respects: (a) The data are presented only in regard to high spinal anesthesia, rather than a mixture of "low" and "high" spinal subjects. (b) All patients were normotensive. (c) The effects of high spinal anesthesia (9 patients) are compared to a group (6 patients) used as double control subjects without anesthesia.

Cerebral blood flow (CBF) was measured by the nitrous oxide method of Kety and Schmidt. Blood samples from the jugular bulb were drawn via an indwelling catheter under fluoroscopic control. High spinal anesthesia was considered induced when the anesthetic level extended above T4, accompanied by increased finger blood flow (Grass oscillographic plethysmograph).

No significant difference was noted in CBF. The mean CBF changed from 40 to 45 ml./100 Gm./min. in the high spinal, and from 45 to 42 ml. in the double control group. Cerebrovascular resistance (CVR) showed a marked decrease in the high spinal group, and virtually no change in the control, as expected from a marked decrease in mean arterial pressure in the former and a slight increase in the latter. Mean $CMRO_2$ went from 2.6 to 3.1 ml./100 Gm./min. in the high spinal group with no change in the con-

trols. Cerebral glucose metabolism, arterial pH, and $pCO_2$ showed no significant variation in either group.

It appears from the results that the cerebral vasodilatory homeostatic responses to the acute reduction in blood pressure accompanying high spinal anesthesia are sufficient to maintain cerebral blood flow and metabolism in the normotensive patient. It is of interest that basal blood flow is maintained only in the brain (except for the skin and muscles, where it is increased); high spinal anesthesia otherwise produces a decrease in blood flow through all other organ systems.

## 67. SEVERE FEBRILE REACTION TO PROBENECID (BENEMID) OCCURRING IN A PATIENT WITH CHRONIC GOUT

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(INTRODUCED BY LOUIS R. LIMARZI, M.D.)

Probenecid (Benemid) reported of value as a uricosuric agent in the treatment of gout was originally introduced by Boger as a blocking agent to increase penicillin and para-aminosalicylic acid levels. He reported a low incidence of toxic manifestations which correlated well with the dosage used. Drug sensitivity tests in the guinea pig failed to produce local or generalized reactions. Certain instances of skin rash in the human being attributed to Benemid sensitivity occurred, but there was difficulty in assigning responsibility in all patients, since both penicillin and PAS which also cause drug rashes had been given concurrently. It was anticipated that some individuals would be sensitive to Benemid. We recently observed a patient who had a severe drug fever in addition to other symptoms while on continuous Benemid therapy. We believe this constitutes a true sensitization reaction.

A 46-year-old man with chronic gout on the tenth day of Benemid treatment (2.0 Gm. daily) developed headache, generalized muscular aching, pruritus, dyspnea, nausea, vomiting, a shaking chill, and a fever of 103.2° F. Therapy was continued for another day during which he developed a macular rash over the entire trunk, had several shaking chills, and a fever to 104.6° F. Benemid was discontinued, and within a day his temperature returned to normal, though other symptoms persisted for several days.

During this period he had received 20 mg. of ACTH intramuscularly in divided doses daily. This dosage was gradually reduced, but before its discontinuance and two weeks after the previous episode he was given a test dose of 0.125 Gm. of Benemid. Within an hour he had a recurrence of the same symptoms with a severe shaking chill and fever to 103° F.

This severe febrile reaction to Benemid occurred following ten days of continuous therapy in spite of concurrent administration of ACTH in small doses. This reaction recurred after two weeks without Benemid therapy following a single test dose of Benemid.

## 68. EFFECTIVENESS OF TREATMENT OF PNEUMOCOCCIC INFECTION IN MICE WITH A COMBINATION OF MULTIPLE DOSES OF AUREOMYCIN AND PENICILLIN ADMINISTERED OVER A THREE-DAY PERIOD

MARK H. LEPPER, M.D., GEORGE GEE JACKSON, M.D., AND HARRY F. DOWLING, M.D., CHICAGO, ILL.

(INTRODUCED BY FORD K. HICK, M.D.)

Antagonism of the curative action of penicillin by Chloromycetin, aureomycin, and terramycin has been demonstrated in a number of experimental

infections when these agents are used in combination and a single dose is given. Administration of multiple doses, as used in clinical practice, has been reported to nullify this effect, yet a clinical series of patients with pneumococcal meningitis received multiple doses of combination therapy and extreme antagonism was observed. In the present studies the effectiveness of combination therapy under a multiple dose regimen in pneumococcal infections in mice has been tried.

Aureomycin has been shown to be antagonistic to penicillin when administered in single doses in experimental pneumococcal infections in mice. In this study the incidence and occurrence of aureomycin antagonism for penicillin when a multiple dose schedule is used have been investigated.

Forty experiments have been performed in which experimental pneumococcal infections in mice have been treated with multiple doses of aureomycin and penicillin over a three-day period, begun both before and after initiating the infection. In eighteen of these there was evidence of antagonism, and in six the magnitude of the difference was sufficient to be of statistical significance within the individual experiment.

In fifteen experiments there was addition of activity exerted by the drugs given in combination, and in four of these the figures for individual experiments were statistically significant. In seven there was no demonstrable addition or antagonism. These results will be compared with differences observed following single doses of combined treatment in pneumococcal infection.

Factors which seem important in determining whether the effect of the combined drugs is additive, antagonistic, or the same as the effect of a single antibiotic are the protective level of the individual drugs, the time relationships of administration, and the duration of the infection without treatment or the magnitude of the initial infection. These will be discussed.

## 69. TREATMENT OF TUBERCULOUS MENINGITIS IN SEVENTEEN PATIENTS WITH ISONICOTINIC ACID HYDRAZIDE (ISONIAZID)

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(INTRODUCED BY FORD K. HICK, M.D.)

Results of treatment of seventeen patients with tuberculous meningitis during the past six months with isonicotinic acid hydrazide, isoniazid (INA) (alone in nine patients, and with streptomycin in eight patients) are compared with sixty patients treated in the preceding two years with streptomycin and Promizole, and/or para-aminosalicylic acid (PAS).

The most favorable results previously obtained were in patients with streptomycin intramuscularly in combination with either Promizole or PAS, or both, along with daily intrathecal administration of streptomycin. This group, which is used as a control group, consists of 37 patients among whom eight (22 per cent) are alive and doing well seven to twenty-seven months after the beginning of treatment. In this group the death rate was 60 per cent by the end of the first month of treatment and 65 per cent by the end of the second month.

In the INA group, five (29 per cent) among 17 died in the first month and eight (47 per cent) by the end of the second month. Two patients are still alive in the second month, three in the third month, three in the fourth month, and one each in the fifth and sixth months of therapy. All patients

who have lived past the second month are in good condition clinically, and spinal fluid findings have improved markedly. The response to therapy clinically or by laboratory criteria has been much more rapid in the INA group. A comparison will be made of duration of fever, mental abnormality and spinal fluid changes in the two groups which will illustrate this. There has been no difference at the present time between patients who received INA only or with streptomycin.

The more favorable results in the INA group cannot be explained by difference in age, race, or degree of illness at the time treatment was started, since there is little or no weighing of the groups in respect to these factors.

## 70. THE INFLUENCE OF RELATIVE HUMIDITY ON THE SURVIVAL OF INFECTIOUS AGENTS DEPOSITED UPON SURFACES

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The survival of infectious agents upon surfaces may be of considerable importance in the transmission of infection, and it has been observed that the viable microorganisms deposited upon surfaces may be redispersed into the air and give rise to inhalation infection or they may cause contact infection via hand to mouth transfer. Because of these considerations, studies were initiated to determine the pattern of survival of specific infectious agents deposited upon surfaces and maintained at room temperature but under conditions of controlled relative humidity. The pattern of survival was determined for Group C hemolytic streptococci, Type I pneumococci, *Staphylococcus albus, E. coli,* and influenza virus (PR8 strain).

The following procedure was devised in an effort to reproduce the events which occur when an infected droplet is expelled from the nasopharynx and falls upon a surface subsequently evaporating until equilibrium is attained with the ambient relative humidity. Inocula, in a volume of 0.05 ml. of the appropriate standard culture (approximately $10^6$ viable microorganisms) in serum dextrose broth or lung virus suspension, were placed upon chemically clean, sterile glass slides which then were immediately placed inside sealed desiccator jars containing supersaturated solutions of various salts which would produce and maintain the desired water vapor content in the enclosed jars. For these studies, relative humidities of 16, 52, 75, and 95 per cent were selected. At appropriate time intervals the slides were removed from the jar and placed in 10 ml. of sterile heart infusion broth, agitated for two minutes, and the number of surviving microorganisms determined by plating or, in the case of influenza virus, by intranasal instillation into white mice.

At 16 per cent relative humidity, survival was maximum for all agents tested and in the case of Group C hemolytic streptococci the population declined less than one logarithmic unit over a fourteen-month period, while Type I pneumococci survived in large numbers ($10^3$) for eleven months at this humidity. Influenza virus retained infectiousness after one hundred seevnty days' exposure to 16 per cent relative humidity. As the humidity was raised above 16 per cent, survival decreased progressively. It was noted that both *Staphylococcus albus* and *E. coli* were able to increase between ten and one hundred fold during the first week of exposure to 95 per cent relative humidity and thereafter died off rapidly.

## 71. ABSORPTION AND URINARY EXCRETION OF ORALLY ADMINISTERED RADIOACTIVE IODINATED HUMAN SERUM ALBUMIN IN HUMAN BEINGS

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Fourteen patients without obvious metabolic disturbances and three poorly nourished patients received 50 to 100 microcuries of radioactive human serum albumin homogenized with powdered Protenum (1 Gm. per kilogram body weight) diluted to one pint with whole milk. This mixture was ingested within five to ten minutes. Blood samples were withdrawn at hourly intervals to the sixth hour following the ingestion of the protein mixture. In all instances radioactivity of the protein mixture and the plasma samples was counted using a Geiger-Müller tube. Urine was collected quantitatively during the first twenty-four hours following administration in five of the patients, and radioactivity was similarly determined in these samples. All patients were prepared by administration of Lugol's solution prior to the experiment.

Radioactivity is here expressed as per cent of total activity ingested which is present in the circulating blood plasma. Total plasma volumes were estimated on the basis of 49.6 c.c. per kilogram of body weight. This estimate is based on data obtained in our laboratory. At one hour following administration of the mixture, an average of 5.33 per cent of ingested radioactivity was present in the circulating plasma. The maximum amount (9.30 per cent) was observed at the end of the third hour. A sloping plateau was present to the end of the sixth hour, at which time 7.41 per cent remained in the plasma. At twenty-four hours, there was a significant decrease in the average plasma activity to 2.50 per cent. No difference between normal and undernourished patients was apparent. Statistical evaluation of these data indicate that they are significant.

Urinary excretion of activity was observed to fall between 41 per cent and 74 per cent, with an average of 57.5 per cent of the total dose administered. This was significantly higher than urinary excretion of radioactivity following the intravenous administration of iodinated human serum albumin.

These data cannot yet be fully evaluated from a physiological standpoint, but further studies are in progress to aid in such analyses. It is felt that this technique will be a useful tool in the study of protein metabolism in human beings.

## 72. THE VALUE OF THYROTROPIN IN THE DIAGNOSIS OF OBSCURE HYPOTHYROIDISM

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(INTRODUCED BY JOSEPH M. HAYMAN, JR., M.D.)

The question of the presence of hypothyroidism, particularly in patients who have been taking thyroid medication, is a common problem in clinical medicine. In many such cases the usual tests for thyroid function are not helpful unless the patient is followed for a period of months.

It has been found that a single injection of thyrotropic hormone (TSH) produces a significant increase in the rate of uptake of $I^{131}$ in individuals with

normal thyroid function whether or not they have been taking thyroid. This has been used as the basis for a test for detecting obscure cases of hypofunction of the gland. The test consists of a comparison of the three-hour thyroid uptake of tracer doses of $I^{131}$ before and twenty-one hours after the intramuscular injection of a single dose of TSH. The response to doses varying between 5 and 20 mg. has been studied in 50 instances. Euthyroid individuals, even after having taken as much as 0.26 Gm. of thyroid for over eighteen months, uniformly showed a significant response to TSH, whereas those with primary hypothyroidism showed little if any increase in uptake. A single case of panhypopituitarism had a good response.

This relatively simple test is of particular value because of its ability to evaluate thyroid function without discontinuing thyroid medication.

*(To be concluded in the December issue.)*

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## Erratum

In the August, 1952, issue of the JOURNAL on page 201, paragraph 3, the second line should read "the solutions above, namely, 1:800 (weight:weight), 1:200, 1:400, and 1:8,000, respectively."